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(54) **Escape mutant of the surface antigen of hepatitis B virus**

(57) The invention provides an isolated mutant hepatitis B surface antigen protein which comprises an amino acid sequence of a surface antigen protein of hepatitis B virus which infects humans, in which the amino acid at position 121 is not cysteine and at least one of the amino acids at positions 120, 122, 123, 147, or 149 is not a conserved amino acid for its position. The invention also provides a method for detecting in a sample a mutant hepatitis B surface antigen protein or a particle containing the protein.

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## Description

FIELD OF THE INVENTION

5 [0001] The present invention relates to an escape mutant of the surface antigen of the hepatitis B virus.

BACKGROUND OF THE INVENTION

10 [0002] Serum from patients infected with hepatitis B virus (HBV) commonly have three distinct structures that contain the hepatitis B surface antigen (HBsAg): Dane particles, spherical particles, and filamentous particles. Dane particles are spheres that are 42 nm in diameter with a core that is 28 nm in diameter. The spherical particles have a diameter of about 22 nm. Filamentous particles have a diameter of about 22 nm and a length from about 50 nm to about 230 nm. [0003] The particles contain three glycoproteins designated the major, middle, and large proteins. The hepatitis B surface antigen open reading frame of HBV-DNA is divided into three regions, pre-S1, pre-S2, and S. This open reading frame encodes the major, middle, and large proteins. The complete amino acid sequence for the major protein is given in Valenzuela et al., *Nature*, 280:815-819 (1979). The amino acid sequence in Valenzuela et al. is 226 amino acids long and the amino acid positions referred to in this application refer to the amino acid sequence disclosed in Valenzuela et al. HBsAg contains several antigenic determinants, the most important of these are the a determinant, the d/y determinant, and the w/r determinant.

20 [0004] Hepatitis B vaccines have been used extensively in humans in recent years. A typical vaccine, e.g., RECOM-BIVAX HB vaccine, available from Merck, contains HBsAg of the adw subtype that has been produced recombinantly in yeast. As the human population becomes vaccinated, the virus is put under pressure to evolve around the vaccine. Mutants that evolve in response to the vaccine are termed "escape mutants." Current vaccines may not be effective against these escape mutants. Also, some current immunodiagnostic tests may not detect these escape mutants.

25 [0005] Certain HBV escape mutants have been reported previously. A mutation at position 145 from glycine to arginine was reported by Carman et al., *Lancet*, 336:325-329 (1990). Also, a mutation where additional amino acids were inserted after amino acid 122 was reported in WO 95/21189. Other mutants are described in McMahon et al., *Hepatology*, 15(5):757-766 (1992). In addition, studies have been conducted on artificial mutant proteins. An example of this type of study is Mangold et al., *Virology*, 211:535-543 (1995). Mangold et al. made various mutations at positions 30 121, 124, 137, 139, 147, and 149.

[0006] The invention provides isolated mutant hepatitis B surface antigen proteins, fragments thereof, and particles containing these proteins. These mutant proteins, fragments, and particles can be used in improved vaccines and these mutant proteins, fragments, and particles as specific binding agents to them can be used in improved immunoassays.

35 SUMMARY OF THE INVENTION

[0007] The invention provides an isolated mutant hepatitis B surface antigen protein which comprises an amino acid sequence of a surface antigen protein of hepatitis B virus which infects humans, in which the amino acid at position 121 is not cysteine and at least one of the amino acids at positions 120, 122, 123, 147, or 149 is not a conserved amino acid 40 for its position. The invention also provides an isolated mutant hepatitis B surface antigen protein which comprises an amino acid sequence of a surface antigen protein of hepatitis B virus which infects humans, in which the amino acid at position 122 is not a conserved amino acid for its position.

[0008] The invention also provides a vaccine which comprises an immunogenic amount of a mutant hepatitis B surface antigen protein, a fragment thereof, or a particle containing the protein, in a pharmaceutically acceptable carrier. 45 The invention also provides specific binding agents which specifically recognize a mutant hepatitis B surface antigen protein, a fragment thereof, or a particle containing the protein.

[0009] The invention also provides a method for detecting in a sample a mutant hepatitis B surface antigen protein or a particle containing the protein comprising: (a) contacting a sample with a specific binding agent which specifically recognizes a mutant surface antigen protein of the invention under conditions suitable for binding to occur; and (b) measuring the extent of the binding of the specific binding agent, wherein the extent of the binding correlates to the presence or amount of mutant hepatitis B surface antigen protein or a particle containing the protein in the sample; to determine the presence or amount of mutant hepatitis B surface antigen protein or a particle containing the protein in the sample. Methods for calibrating a method for determining the amount of a mutant hepatitis B surface antigen protein or a particle containing the protein, in a sample and for testing the binding affinity of a specific binding agent a mutant hepatitis B 55 surface antigen protein, a fragment thereof, or a particle containing the protein, are also provided.

[0010] The invention provides a method of detecting in a sample a nucleic acid sequence coding for a mutant hepatitis B surface antigen protein comprising: (a) providing a sample suspected of containing a nucleic acid sequence coding for a mutant hepatitis B surface antigen protein of the invention; and (b) detecting the nucleic acid sequence coding for

the mutant hepatitis B surface antigen protein.

[0011] Additional features and advantages of the invention are set forth in the description which follows and in part will be apparent from the description. The objectives and other advantages of the invention will be realized and attained by the isolated mutant hepatitis B surface antigen proteins, fragments thereof, and particles containing these proteins, specific binding agents thereto, and immunoassays and vaccines using these proteins, fragments thereof, and particles containing these proteins and/or specific binding agents as particularly pointed out in the written description, claims, and appended drawings. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0012]

Fig. 1 depicts the partial amino acid sequence of the CTS mutant hepatitis B surface antigen protein.

Fig. 2 compares the partial amino acid sequence of the CTS mutant hepatitis B surface antigen protein to some other HBV subtypes.

Fig. 3 compares the partial amino acid sequence of the CTS mutant hepatitis B surface antigen protein to some other HBV subtypes.

Figs. 4 and 5 depict phylogenetic subtype groupings and distances for the mutant HBsAg-CTS and for twenty subtypes of the Hepatitis B virus.

Fig. 6 is a graphical representation of the data of Table 8, plotting percent neutralization as a function of the ratio of antibody potency to antigen concentration.

Fig. 7 is a graphical representation of the data of Table 9, plotting percent neutralization as a function of the ratio of antibody potency to antigen concentration.

Fig. 8 is a graphical representation of the data of Table 10, plotting percent neutralization as a function of the ratio of antibody potency to antigen concentration.

[0013] In Figs. 6 to 8 the solid line and squares represent the antibody response to natural HBsAg and the dashed line and diamonds represent the antibody response to the mutant HBsAg.

## DETAILED DESCRIPTION OF THE INVENTION

[0014] The invention provides an isolated mutant hepatitis B surface antigen protein which comprises an amino acid sequence of a surface antigen protein of hepatitis B virus which infects humans, in which the amino acid at position 121 is not cysteine and at least one of the amino acids at positions 120, 122, 123, 147, or 149 is not a conserved amino acid for its position. In one embodiment, the isolated mutant hepatitis B surface antigen protein displays an antigenicity different from that of wild-type hepatitis B surface antigen protein. In another embodiment, at least one of the amino acids at positions 120, 122, or 123 is not a conserved amino acid for its position; in still another embodiment, at least one of the amino acids at positions 147 or 149 is not a conserved amino acid for its position.

[0015] In one embodiment of the invention, at least two of the amino acids at positions 120, 122, 123, 147, or 149 are not conserved amino acids for their positions. In other embodiments, at least three, at least four, or all five of the amino acids at positions 120, 122, 123, 147, or 149 are not conserved amino acids for their positions.

[0016] In other embodiments, the conserved cysteine at position 121 is replaced by tyrosine; the conserved proline at position 120 is replaced by alanine; the conserved lysine or arginine at position 122 is replaced by glutamine; and/or the conserved threonine at position 123 is replaced by proline. In one embodiment, the conserved proline at position 120 is replaced by alanine, the conserved lysine or arginine at position 122 is replaced by glutamine, and the conserved threonine at position 123 is replaced by proline.

[0017] In another embodiment, the amino acid at position 127 is not a conserved amino acid for its position; preferably, the conserved proline at position 127 is replaced by isoleucine. In another embodiment, the amino acid at position 117 is not a conserved amino acid for its position; preferably, the conserved serine at position 117 is replaced by arginine. In another embodiment, the amino acid at position 96 is not a conserved amino acid for its position; preferably, the conserved valine at position 96 is replaced by alanine. In another embodiment, the amino acid at position 113 is not a conserved amino acid for its position; preferably, the conserved serine or threonine at position 113 is replaced by leucine.

[0018] In another embodiment, the amino acid at position 133 is not a conserved amino acid for its position; preferably, the conserved methionine at position 133 is replaced by threonine. In another embodiment, the amino acid at position 140 is not a conserved amino acid for its position; preferably, the conserved threonine at position 140 is replaced by isoleucine. In another embodiment, the amino acid at position 147 is not a conserved amino acid for its position; preferably, the conserved cysteine at position 147 is replaced by tyrosine. In another embodiment, the amino acid at posi-

tion 149 is not a conserved amino acid for its position; preferably, the conserved cysteine at position 149 is replaced by tyrosine.

[0019] In another embodiment, the amino acids at positions 147 and 149 are not conserved amino acids for their positions. In another embodiment, the conserved cysteines at position 121, 147, and 149 are replaced by tyrosines. In still another embodiment, the amino acid at position 145 is the conserved glycine. In another embodiment, the amino acids at positions 137, 138, and 139 are all conserved cysteines. In another embodiment, the amino acid at position 124 is the conserved cysteine.

[0020] The invention provides an isolated mutant hepatitis B surface antigen protein which comprises an amino acid sequence of a surface antigen protein of hepatitis B virus which infects humans, in which the amino acid at position 122 is not a conserved amino acid for its position. In one embodiment, the isolated mutant hepatitis B surface antigen protein displays an antigenicity different from that of wild-type hepatitis B surface antigen protein. In another embodiment, the conserved lysine or arginine at position 122 is replaced by glutamine.

[0021] In embodiments of the invention, the isolated mutant hepatitis B surface antigen protein is the major protein of hepatitis B surface antigen, the middle protein of hepatitis B surface antigen, the large protein of hepatitis B surface antigen. In another embodiment of the invention the protein includes a pre-S sequence. In other embodiments of the invention the protein includes a portion of or a complete pre-S1 sequence or a portion of or a complete pre-S2 sequence.

[0022] In one embodiment of the invention, the isolated mutant hepatitis B surface antigen protein comprises the following sequence of amino acids at positions 117 to 123: Arg-Thr-Gly-Ala-Tyr-Gln-Pro (SEQ ID NO: 28). In another embodiment, the isolated mutant hepatitis B surface antigen protein comprises the following sequence of amino acids at positions 113 to 133: Leu-Ser-Thr-Ile-Arg-Thr-Gly-Ala-Tyr-Gln-Pro-Cys-Thr-Thr-Ile-Ala-Gln-Gly-Thr-Ser-Thr (SEQ ID NO:29). In another embodiment, the isolated mutant hepatitis B surface antigen protein comprises the following sequence of amino acids at positions 140 to 147: Ile-Lys-Pro-Ser-Asp-Gly-Asn-Tyr-Thr-Tyr (SEQ ID NO:30). In one embodiment, the isolated mutant hepatitis B surface antigen protein comprises the following sequence of amino acids at positions 117 to 123: Arg-Thr-Gly-Ala-Tyr-Gln-Pro (SEQ ID NO:28) and the following sequence of amino acids at positions 140 to 147: Ile-Lys-Pro-Ser-Asp-Gly-Asn-Tyr-Thr-Tyr (SEQ ID NO:30). In another embodiment, the isolated mutant hepatitis B surface antigen protein comprises the following sequence of amino acids at positions 117 to 149: Arg-Thr-Gly-Ala-Tyr-Gln-Pro-Cys-Thr-Thr-Ile-Ala-Gln-Gly-Thr-Ser-Thr-Tyr-Pro-Ser-Cys-Cys-Cys-Ile-Lys-Pro-Ser-Asp-Gly-Asn-Tyr-Thr-Tyr (SEQ ID NO:31). In another embodiment, the isolated mutant hepatitis B surface antigen protein comprises the sequence of amino acids listed in SEQ ID NO:2 (see below).

[0023] The invention provides an isolated mutant hepatitis B particle which comprises a mutant hepatitis B surface antigen protein of the invention. The invention also provides fragments of an isolated mutant hepatitis B surface antigen protein of the invention. When used in this application, a "fragment" of a mutant hepatitis B surface antigen protein means a fragment that comprises a sequence of at least thirty amino acids containing the sequence of amino acids at positions 120 to 149. A preferred fragment is an isolated fragment of a mutant hepatitis B surface antigen protein in which the isolated fragment comprises a sequence of at least thirty amino acids, said sequence comprising the amino acids at positions 120 to 149, in which the amino acid at position 121 is not cysteine and at least one of the amino acids at positions 120, 122, 123, 147, or 149 is not a conserved amino acid for its position. Another preferred fragment is an isolated fragment of a mutant hepatitis B surface antigen protein in which the isolated fragment comprises a sequence of at least thirty amino acids, said sequence comprising the amino acids at positions 120 to 149, in which the amino acid at position 122 is not a conserved amino acid for its position.

[0024] The invention also provides a composition which comprises a mutant hepatitis B surface antigen protein of the invention, a fragment thereof, or a particle containing the protein, and is substantially blood-free.

[0025] The invention provides a vaccine which comprises an immunogenic amount of a mutant hepatitis B surface antigen protein of the invention, a fragment thereof, or a particle containing the protein, in a pharmaceutically acceptable carrier.

[0026] The invention provides a specific binding agent which specifically recognizes a mutant hepatitis B surface antigen protein of the invention, a fragment thereof, or a particle containing the protein, wherein the specific binding agent was specifically raised against the mutant hepatitis B surface antigen protein, a fragment thereof, or a particle containing the protein. In one embodiment, the specific binding agent does not substantially bind native HBV with any of the following phenotypes: ayw, ayr, adr, or adw. In another embodiment, the specific binding agent substantially binds native HBV with any of the following phenotypes: ayw, ayr, adr, or adw. In one embodiment the specific binding agent recognizes a sequential epitope; in another embodiment the specific binding agent recognizes a conformational epitope. The specific binding agent may be a monoclonal antibody, a fragmentary monoclonal antibody, or a polyclonal antibody.

[0027] The invention provides a secreting hybridoma of a monoclonal antibody of the invention.

[0028] The invention provides a control sample comprising a known amount of a mutant hepatitis B surface antigen protein, a fragment thereof, or a particle containing the protein.

[0029] The invention provides a method for detecting in a sample a mutant hepatitis B surface antigen protein or a

particle containing the protein comprising: (a) contacting a sample with a specific binding agent which specifically recognizes a mutant surface antigen protein of the invention under conditions suitable for binding to occur; and (b) measuring the extent of the binding of the specific binding agent, wherein the extent of the binding correlates to the presence or amount of mutant hepatitis B surface antigen protein or a particle containing the protein in the sample; to determine the presence or amount of mutant hepatitis B surface antigen protein or a particle containing the protein in the sample. In one embodiment, the correlation between the extent of the binding and the presence or amount of mutant hepatitis B surface antigen protein or a particle containing the protein in the sample has been determined using control samples containing known amounts of the mutant hepatitis B surface antigen protein, a fragment thereof, or a particle containing the protein. In another embodiment, the specific binding agent was specifically raised against the mutant hepatitis B surface antigen protein or a particle containing the protein. In another embodiment, the method comprises a non-competitive assay. In still another embodiment, the assay comprises a second specific binding agent which has a label; the label can be a radioactive label, an enzyme label, a fluorescent label, a chemiluminescent label, a bioluminescent label, or an epifluorescent label. In another embodiment, the method comprises an agglutination assay; in still another embodiment, the method comprises a competitive assay. The method can comprise using a labeled substrate that cross-reacts with the mutant hepatitis B surface antigen protein or particle containing the protein. The sample can be a blood sample or a vaccine.

[0030] The invention provides a method for calibrating an assay for a mutant hepatitis B surface antigen protein or a particle containing the protein comprising: (a) providing two or more control samples comprising known amounts of a mutant hepatitis B surface antigen protein of the invention, a fragment thereof, or a particle containing the protein; (b) testing each of the control samples in the assay and recording the signal that each control sample produced; and (c) producing a calibration curve that correlates the signal produced with the amount of mutant hepatitis B surface antigen protein, the fragment thereof, or the particle containing the protein in a sample.

[0031] The invention provides a method of testing the binding affinity of a specific binding agent comprising: (a) providing a control sample comprising a known amount of a mutant hepatitis B surface antigen protein of the invention, a fragment thereof, or a particle containing the protein; (b) contacting the control sample with a specific binding agent under conditions suitable for binding to occur; and (c) measuring the extent of the binding of the specific binding agent; to determine the binding affinity of the specific binding agent to the mutant hepatitis B surface antigen protein, the fragment thereof, or the particle containing the protein. In one embodiment, the binding affinity is compared with a predetermined minimum value for the binding affinity. In another embodiment, this comparison is part of a quality control procedure for producing a diagnostic immunoassay kit. In a preferred embodiment, the specific binding agent is a monoclonal antibody or a fragmentary monoclonal antibody.

[0032] The invention provides a diagnostic immunoassay kit comprising a specific binding agent of the invention. The invention also provides a diagnostic immunoassay kit comprising a control sample of the invention.

[0033] The invention provides a mutant hepatitis B surface antigen protein of the invention, a fragment thereof, or a particle containing the protein, which has been recombinantly produced. In one embodiment, the protein, particle, or fragment has been recombinantly produced in a yeast, bacterium, or mammalian cell.

[0034] The invention provides isolated DNA which codes for a mutant hepatitis B surface antigen protein of the invention, a fragment thereof, or a particle containing the protein. In one embodiment, the isolated DNA comprises a sequence which codes for the amino acid sequence of SEQ ID NO: 2. In another embodiment, the isolated DNA comprises a sequence which codes for the following amino acid sequence: Arg-Thr-Gly-Ala-Tyr-Gln-Pro-Cys-Thr-Ile-Ala-Gln-Gly-Thr-Ser-Thr-Tyr-Pro-Ser-Cys-Cys-Cys-Ile-Lys-Pro-Ser-Asp-Gly-Asn-Tyr-Thr-Tyr (SEQ ID NO:31).

[0035] The invention provides a vector comprising DNA which codes for a hepatitis B surface antigen protein of the invention, a fragment thereof, or a particle containing the protein.

[0036] The invention provides a method of detecting in a sample a nucleic acid sequence coding for a mutant hepatitis B surface antigen protein comprising: (a) providing a sample suspected of containing a nucleic acid sequence coding for a mutant hepatitis B surface antigen protein of the invention; and (b) detecting the nucleic acid sequence coding for the mutant hepatitis B surface antigen protein. In a preferred embodiment, prior to the detecting step (b) at least a portion of the nucleic acid sequence is amplified. In another embodiment, the detecting step is performed by hybridizing the amplified nucleic acid with at least a portion of a nucleotide probe comprising a nucleotide sequence complementary to the amplified mutant hepatitis B nucleic acid; and detecting the probe. In another embodiment the probe is labeled. In still another embodiment, the hybridized probe and amplified nucleic acid complex is detected by a specific binding agent which is specific to double-stranded DNA; the specific binding agent preferably is a monoclonal antibody or a fragmentary monoclonal antibody. In another embodiment, the detecting step is performed by separating the amplified nucleic acid according to nucleotide sequence length and detecting nucleotide sequences corresponding to amplified hepatitis B nucleic acid.

[0037] The invention provides a nucleotide probe which comprises a nucleotide sequence selected from:

sense probe #1 (from nt 491 to nt 526):

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5'-GGA YTM TCG ACC ATC MGC ACG GGA GCA TAC CAA CCC-3' (SEQ ID NO:34);

antisense probe #2 (from nt 526 to nt 491):

5'-GGG TTG GTA TGC TCC CGT GCK GAT GGT CGA KAR TCC-3' (SEQ ID NO:35);

sense probe #3 (from nt 500 to nt 523):

5'-ACC ATC MGC ACG GGA GCA TAC CAA-3' (SEQ ID NO:36);

antisense probe #4 (from nt 523 to nt 500):

5'-TTG GTA TGC TCC CGT GCK GAT GGT-3' (SEQ ID NO:37);

sense probe #5 (from nt 488 to nt 511):

5'-CCA GGA YTM TCG ACC ATC MGC ACG-3' (SEQ ID NO:38);

antisense probe #6 (from nt 514 to nt 491):

5'-TCC CGT GCK GAT GGT CGA KAR TCC-3' (SEQ ID NO:39);

sense probe #7 (from nt 515 to nt 538):

5'-GCA TAC CAA CCC TGC ACG ACT ATT G-3' (SEQ ID NO:40);

antisense probe #8 (from nt 538 to nt 515):

5'-C AAT AGT CGT GCA GGG TTG GTA TGC-3' (SEQ ID NO:41);

sense probe #9 (from nt 573 to nt 605):

5'-GT ATC AAA CCT TCG GAC GGA AAT TAC ACC TAT A-3' (SEQ ID NO:42);

sense probe #10 (from nt 584 to nt 605):

5'-TCG GAC GGA AAT TAC ACC TAT A-3' (SEQ ID NO:43);

antisense probe #11 (from nt 616 to nt 595):

5'-TGG GAT GGG AAT ATA GGT GTA A-3' (SEQ ID NO:44);

or a nucleotide sequence having substantially the same nucleotide sequence and having substantially the same hybridization activity of any of the listed nucleotide sequences. In a preferred embodiment, the probe consists essentially of one of the sequences listed above. In a preferred embodiment, these probes are used in the methods and kits of the invention. These probes may further comprise a detectable label. Primers appropriate for use with these probes are apparent to one of skill in the art.

[0038] The invention provides a kit for the detection of a nucleic acid sequence coding for a mutant hepatitis B surface antigen protein of the invention comprising: at least one reagent comprising nucleotide primer capable of annealing to and priming for amplification with nucleic acid characteristic for hepatitis B surface antigen; and at least one reagent comprising a nucleotide probe capable of hybridizing with the amplified mutant hepatitis B surface antigen nucleic acid.

[0039] Hepatitis B surface antigen DNA was cloned and sequenced from a serum sample taken from a kidney transplant patient (designated CTS) who tested positive for hepatitis B surface antigen (HBsAg) in an ETI-MAK-3 assay (Sorin Diagnostics, Saluggia, Italy) and negative in an AXSYM-HBsAg assay (Abbott Laboratories). The hepatitis B surface antigen designated CTS was not detected by the Abbott assay due to a mutation of the hepatitis B virus. Using an assay that provides false negative indications could result in a delay in providing effective treatment to a patient infected by the mutation or allow transmission of the mutant HBV from a blood donor to a recipient.

[0040] When the cloned nucleotide sequence is translated, its product displays a large number of mutations which are believed to determine differences in the secondary and tertiary structure of the antigen. Such mutations also determine differences in the antigenicity of the protein.

[0041] This mutant HBsAg-CTS is most closely homologous to native HBsAg of the ayw subtype. The relative homology suggests that the HBsAg-CTS evolved from the ayw subtype. Similarities notwithstanding, HBsAg-CTS possesses certain characteristics which suggest that it is phylogenically removed from the ayw subtype in a significant way. In particular, the d/y determinant, identified by the presence of a basic amino acid (Arg (R) or Lys (K)) in position 122 is missing in HBsAg-CTS. Instead, the mutant HBsAg-CTS has a glutamine (Gln (Q)) residue in this position. Notably, however, the w/r determinant is maintained, as the residue in position 160 is a lysine (Lys (K)). The w/r determinant is identified by the presence of a basic amino acid (Arg (R) or Lys (K)) in position 160.

[0042] Other significant differences can be found as well. For example, in HBsAg-CTS, the residues at positions 121,

147, and 149, which are commonly cysteine (Cys (C)) have been replaced by tyrosine (Tyr (T)). Notably, positions 121, 147, and 149 are considered to be very important for the folding of the  $\alpha$  determinant of HBsAg into a double loop, stabilized by disulfide bridges, in the relevant portion of the protein. Such stabilized double loops are a characteristic of the  $\alpha$  determinant and are believed to influence the antigenicity of the antigen. More importantly, the stabilized double loop at the  $\alpha$  determinant of HBsAg represents the immunodominant epitope against which a host can produce antibodies. Because they have an abnormal conformation of the immunodominant epitope, mutant strains of the Hepatitis B virus may be able to evade detection using conventional assay methods.

[0043] It should be noted, however, that even lacking the double loop immunodominant epitope, it is still possible to have antibodies which bind to the mutant HBsAg via a conformational epitope. Also, antibodies may bind the mutant HBsAg via s sequential epitope. Likewise, a monoclonal antibody having an affinity to mutant HBsAg may be used in the manufacture of assays capable of detecting and quantifying mutant strains of the Hepatitis B virus and in vaccines capable of raising an immune response against such mutant strains of the Hepatitis B virus.

[0044] The HBsAg-CTS includes additional mutations. By analyzing the alignment of the amino acid sequence of HBsAg-CTS with amino acid sequences belonging to different Hepatitis B virus subtypes, eight other mutations appear in positions which are generally well conserved among the various subtypes. These additional mutations (at positions 96, 113, 117, 120, 123, 127, 133, 140, 147, and 149) are shown in Table 1 in which the conserved amino acids are those commonly found at the particular position among the various subtypes, and the substituted amino acids are those that may be found at those positions in HBsAg-CTS.

TABLE 1

Position	Conserved Amino Acid	Substituted Amino Acid
96	Val	Ala
113	Ser/Thr	Leu
117	Ser	Arg
120	Pro	Ala
121	Cys	Tyr
122	Lys	Gln
123	Thr	Pro
127	Pro	Ile
133	Met	Thr
140	Thr	Ile
147	Cys	Tyr
149	Cys	Tyr

[0045] The DNA that codes for the mutant HBsAg-CTS comprises the following sequence of 276 nucleotides:

TGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTC  
 TTGTTGGCTCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCA  
 GGACTCTCGACCATCCGCACGGGAGCATACCAACCCTGCACGACTATTGCTCAA  
 GGAACCTCTACGTATCCCTCCTGTTGCTGTATCAAACCTTCGGACGGAAATTAC

ACCTATATTTCCCATCCCATCATCTGGGCTTTTCGGAAAAYYCCTATGGGAGTGG  
 GCCTCA (SEQ ID NO:1)

This sequence of 276 nucleotides encodes the following sequence of 92 amino acids:

Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu  
 1 5 10  
 5 Leu Cys Leu Ile Phe Leu Leu Ala Leu Leu Asp Tyr Gln  
 15 20 25  
 Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Leu Ser  
 10 30 35  
 Thr Ile Arg Thr Gly Ala Tyr Gln Pro Cys Thr Thr Ile  
 40 45 50  
 15 Ala Gln Gly Thr Ser Thr Tyr Pro Ser Cys Cys Cys Ile  
 55 60 65  
 Lys Pro Ser Asp Gly Asn Tyr Thr Tyr Ile Pro Ile Pro  
 70 75  
 20 Ser Ser Trp Ala Phe Gly Lys Phe Leu Trp Glu Trp Ala  
 80 85 90  
 Ser (SEQ ID NO:2)

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In SEQ ID NO:2 the amino acid positions are numbered sequentially beginning with "1". To convert these positions to the standard positions in the HBsAg genome, add "75" to each position. This transformation is necessary for SEQ ID NOS:2-6. To convert the positions in SEQ ID NOS:7-27 to the standard positions in the HBsAg genome, add "33" to each position. In Fig. 1, SEQ ID NO:2 is presented using single letter symbols for the amino acids. Both the nucleotide sequence (SEQ ID NO:1) and the amino acid sequence (SEQ ID NO:2) are only portions of the entire sequences; the DNA coding for the major protein of HBsAg has a total of 226 amino acids.

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**[0046]** Mutations of the HBsAg-CTS are readily apparent when the 92 amino acid sequence (SEQ ID NO:2) is aligned with different subtypes of HBsAg (ayw 3, adr, adw 2, and ayw). Partial sequences for these different subtypes are given in the attached sequence listing. (SEQ ID NO:3 is from HBVAYW3, SEQ ID NO:4 is from HBVADR, SEQ ID NO:5 is from HBVADW2, and SEQ ID NO:6 is from HBVAYW.) For ease of comparison, the sequences are compared with SEQ ID NO:2 in Fig. 2, using the single letter symbols for amino acids. In these alignments, the mutant amino acids are represented in bold and underlined.

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**[0047]** Expansion of the comparison to multiple species within each subtype has been carried out with amino acid sequences that are 154 amino acids in length. In these analyses, HBsAg-CTS is compared to twenty different HBsAg species contained within four different HBsAg subtypes. Partial sequences for these different subtypes are given in the attached sequence listing. SEQ ID NO:7 is from 7AYWS, SEQ ID NO:8 is from 6AYWS, SEQ ID NO:9 is from 3AYWS, SEQ ID NO: 10 is from 2AYWS, SEQ ID NO:11 is from 1AYWS, SEQ ID NO: 12 is from 1ADYWS, SEQ ID NO:13 is from 5AYWS, SEQ ID NO: 14 is from 5ADWS HBVAYW, SEQ ID NO:15 is from 2ADWS, SEQ ID NO: 16 is from 3ADWMUT, SEQ ID NO:17 is from 2ADWMUT, SEQ ID NO: 18 is from 1ADWS, SEQ ID NO:19 is from 4ADWMUT, SEQ ID NO:20 is from 3ADWS, SEQ ID NO:21 is from 1ADWMUT, SEQ ID NO:22 is from 4ADRS, SEQ ID NO:23 is from 1ADRS, SEQ ID NO:24 is from 3ADRS, SEQ ID NO:25 is from 2ADRS, SEQ ID NO:26 is from 4ADWS, and SEQ ID NO:27 is from CTS.

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**[0048]** For ease of comparison the sequences are compared with SEQ ID NO:2 in Fig. 3, using the single letter symbols for amino acids. In these alignments, the mutant amino acids are represented in bold and underlined. The symbol "\*" indicates that a position in the alignment is perfectly conserved in the mutant, and the symbol "." indicates that a position in the alignment is well conserved in the mutant.

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**[0049]** In these 154 amino acid sequences, the mutant HBsAg-CTS had 100 amino acids (64.9%) that were identical ("perfectly conserved") to the amino acids in the corresponding position of each of the other twenty subtypes, and 32 amino acids (20.8%) that were similar ("well conserved") to the amino acids in the corresponding position of the other twenty subtypes.

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**[0050]** Amino acid homology between the inventive isolate and the other subtypes is presented in Table 2. Nucleotide and deduced amino acid sequences were analyzed employing the CLUSTAL program contained in the PC/GENE soft-



ware package (IntelliGenetics, Mountain View, California); the sequences used in the alignments were downloaded from the GenBank Database (accession numbers: J02203, X59795, X65257, X77310, X77308, X65258, J02202, D00329, D00330, X51970, X69798, M54923, S81945, S81946, D16665, X01587, S50225, D23677, D23678, and Z72478).

TABLE 2

	<u>ayw</u>	<u>adw/1</u>	<u>adw/2</u>	<u>adr</u>
CTS	89-91%	82-83%		83-94%
<u>ayw</u>	>96%	89-91.5%		88-92%
<u>adw/1</u>	-	98%	92-93%	-
<u>adw/2</u>	-	-	96%	-
<u>adr</u>	-	89-92%		>96%

[0051] A phylogenetic analysis, comparing the 154 amino acid sequence from HBsAg-CTS (SEQ ID NO:27) to equally-sized fragments of the twenty species (SEQ ID NOS:7-26) was performed using the PHYLIP package (Phylogenetic Inference Package) version 3.5c 1993, directly distributed from Dr. J. Felsenstein, Department of Genetics, University of Washington, Seattle. The clustering of the different subtypes was achieved by performing a bootstrap analysis using the SEQBOOT program, also contained in the PHYLIP package, resampling the data 250 times, in order to eliminate some factors which can have an aspecific influence on the various phylogenetic clusters. The results of the phylogenetic analysis are presented in Figs. 4 and 5. In Fig. 4, which seeks to identify relevant subtypes, it is seen that the HBsAg-CTS mutant is a member of the ayw subtype of HBsAg. In Fig. 5, however, the phylogenetic distance of HBsAg-CTS from the ayw subtype of HBsAg is clearly evident. In FIG. 5, the length of the branch is proportional to the phylogenetic length.

[0052] Phylogenetic distances of the HBsAg-CTS from the different HBsAg subtypes, and of the different HBsAg subtypes from one another, are presented in Table 3.

TABLE 3

	<u>ayw</u>	<u>adw/1</u>	<u>adw/2</u>	<u>adr</u>
CTS	0.097-0.119	0.191-0.200		0.175-0.191
<u>ayw</u>	<0.04	0.089-0.119		0.082-0.127
<u>adw/1</u>	-	<0.0131	0.068-0.082	-
<u>adw/2</u>	-	-	<0.033	-
<u>adr</u>	-	0.082-0.119		<0.033

[0053] The data of Tables 2 and 3 and FIGS. 4 and 5 suggest that HBsAg-CTS evolved from the ayw subtype; however, its phylogenetic distance from the ayw subtype is comparable to distances seen between the various subtypes. Additionally, the phylogenetic distance of the HBsAg-CTS from the other members within the ayw subtype is much greater (0.097-0.119) than the phylogenetic distances existing within each of the various subtypes (<0.04).

[0054] HBsAg-CTS has been confirmed as an escape mutant by means of a neutralization assay following the protocol described in the REAC 801 Confirmatory Test (FDA License No. 89-0376). Three neutralizing solutions were used: (a) human plasma anti-HBsAg positive (used in a kit identified as REAC 801, natural infection, available from Sorin Diagnostics, Saluggia, Italy), (b) human IgG anti-HBsAg positive (Biagini serum, a natural infection material used in passive prophylaxis, available from Farma Biagini S.p.A.), and (c) human serum obtained from a donor after vaccination with ENGERIX-B vaccine, available from SmithKline Beecham.

[0055] A protocol, approved by the U.S. Food and Drug Administration, was used in which a sample was considered neutralized, and thereby confirmed HBsAg positive, when greater than 30% neutralization occurred. Of course, it should be noted that 30% is a relative value which can be influenced by the potency of the neutralizing antibody and the concentration of antigen in the sample. Relative comparisons between the different antigens and antibodies was achieved by normalizing the neutralizing antibodies at different concentrations. These concentrations were expressed in mIU. Although it is more difficult to normalize the concentration of the antigen, it was quantified to a concentration of approximately 1.3 PEI U/mL (PEI is an abbreviation for the Paul Erlich Institut, Germany) for the natural HBsAg subtype

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ad, and to approximately 0.5 PEI U/mL for the mutant (CTS) Under these conditions, it is expected that the antibodies will inhibit the mutant to a much higher degree than the natural species. This is true because of the concentration: there is a higher ratio of antibodies to antigen for the mutant antigen in comparison with the natural one. So because the neutralization of the mutant antigen is lower than the neutralization of the natural antigen, these results show very clearly that the mutant should be introduced both in vaccines and diagnostic reagents.

[0056] The results of the neutralization assays are presented below. In each of the assays, the HBsAg detection was performed using an ETI-MAK-2 PLUS kit, lot PP885 (available from Sorin Diagnostics, Saluggia, Italy); this kit contains REAC 801 Sol. A. Results are shown in Table 4:

TABLE 4

Specimen	REAC 801 Sol. A O.D.
Negative Control (NC)	0.027
Positive Control (PC) (1 UPEI/mL)	1.490
Cut off	0.077
CTS (0.5 UPEI/mL)	0.804
natural HBsAg subtype <u>ad</u> (1.3 UPEI/mL)	1.799

The results reported in Table 4 show the values obtained on the tested specimen by using an HBsAg negative human serum in the neutralization assay (Sol. A of REAC 801) in order to determine the baseline of the HBsAg positive specimen in non-inhibited form, to compute the percent of neutralization.

[0057] The results of the neutralization assays for the three sample solutions are shown in Tables 5 to 7.

TABLE 5

REAC 801 (Human Plasma anti-HBs+)						
Samples	10000 mIU/mL (anti-HBs)	%neu	1000 mIU/mL (anti-HBs)	%neu	100 mIU/mL (anti-HBs)	%neu
CP	0.418	73	1.029	32	1.372	8
CTS	0.385	54	0.782	3	0.825	-3
HBsAg	0.366	81	1.236	32	1.759	2

TABLE 6

hlgG anti-HBs (Biagini Serum)						
Samples	10000 mIU/mL (anti-HBs)	%neu	1000 mIU/mL (anti-HBs)	%neu	100 mIU/mL (anti-HBs)	%neu
CP	0.416	73	0.855	43	1.135	24
CTS	0.356	58	0.610	25	0.677	16
HBsAg	0.354	82	0.931	49	1.329	27

TABLE 7

Vaccinated Serum (ENGERIX-B)						
Samples	10000 mIU/mL (anti-HBs)	%neu	1000 mIU/mL (anti-HBs)	%neu	100 mIU/mL (anti-HBs)	%neu
CP	0.451	71	0.884	41	1.273	15
CTS	0.416	50	0.745	8	1.090	-37
HBsAg	0.251	87	1.048	42	1.594	12

[0058] Finally, the ratios of antibody potency (Ab, expressed in mIU/mL) to antigen concentration (Ag, expressed in PEI U/mL) are shown in Tables 8 to 10 below.

TABLE 8

REAC 801 (Human Plasma anti-HBs+)			
CTS Ab/Ag	% neutralization	natural HBsAg Ab/Ag	% neutralization
20	54	7.7	81
2	3	0.8	32
0.2	-3	0.1	2

TABLE 9

hIgG anti-HBs (Biagini Serum)			
CTS Ab/Ag	% neutralization	natural HBsAg Ab/Ag	% neutralization
20	58	7.7	82
2	25	0.8	49
0.2	16	0.1	27

TABLE 10

Vaccinated Serum (ENGERIX-B)			
CTS Ab/Ag	% neutralization	natural HBsAg Ab/Ag	% neutralization
20	50	7.7	87
2	8	0.8	42
0.2	-37	0.1	12

The results of Tables 8 to 10 are also shown graphically, plotted in Figs. 6 to 8, respectively. The data shown in Tables 8 to 10 and Figs. 6 to 8 demonstrate that HBsAg-CTS is an escape mutant.

[0059] As noted above, the presence of HBsAg-CTS was detected in an ETI-MAK-3 assay, but not detected in an AXSYM-HBsAg assay. This result suggests that the AXSYM-HBsAg assay depends upon the presence of the immunodominant epitope "a" which has a mutated conformation in HBsAg-CTS. In contrast, the ETI-MAK-3 assay detects the

immunodominant epitope "a" in the natural or mutated conformation. It is noted that as used herein, the terms "detect" and "detection" and "determination" in the context of an immunoassay are intended to mean either one or both of determining the presence or the amount of a target species in a sample.

[0060] The ETI-MAK-3 assay provides for the qualitative determination of HBsAg using a direct, non-competitive sandwich assay which employs enzyme linked immunosorbent assay (ELISA) technology. In general terms, a monoclonal antibody having an affinity for HBsAg is bound to a surface, such as the wall of a microtiter well. The antibody is exposed to a sample which may or may not contain HBsAg. If HBsAg is present in the sample, it will become bound to the antibody forming antibody/HBsAg complexes. The surface is then exposed to an enzyme tracer which will bind to the antibody/HBsAg complexes. By detecting the level of bound tracer, it is then possible to determine the level of HBsAg in the sample. One preferred tracer is an anti-HBsAg antibody obtained from sheep to which is bound a horse-radish peroxidase.

[0061] Commercially available assays typically include a variety of reagents necessary to determine the presence of a particular biological material. For example, the ETI-MAK-3 assay includes a microtiter plate having wells coated with a monoclonal antibody having an affinity for HBsAg, an enzyme tracer, positive and negative control solutions, tracer diluent, wash buffer, chromogen solution, substrate solution, and a blocking reagent. These last three reagents are used to properly develop a detectable signal indicative of the presence and level of tracer (and thereby antigen) in the sample.

[0062] The present invention is particularly useful for the manufacture of immunoassay kits for the Hepatitis B virus in that it may be used to ensure that the monoclonal antibody provided in such kits maintains its affinity to HBsAg even in the absence of the immunodominant epitope in its natural conformation. Specifically, mutant Hepatitis B virus may not be detected in assays which rely upon detection of the immunodominant epitope (a) of HBsAg in its natural conformation. Thus, detection of the mutant strain requires the use of an antibody that binds to the mutant antigen. In order to assure that the antibodies used in the immunoassay kits will detect the mutant Hepatitis B virus, the assay manufacturing process requires that the supplied antibodies maintain their affinity for the mutant antigen. This may be achieved in manufacture by exposing antibody specimens to a known calibration standard of the mutant antigen and then detecting the presence and level of the antigen. If the assay provides an accurate determination, then the antibody will be provided in the immunoassay kit. If the assay fails to provide an accurate determination, then the antibody should be replaced with one that meets the immunoassay requirements. In so doing, it is assured that the commercial immunoassay kits will have the ability to detect the presence and level of HBsAg in a sample, even if the HBsAg is of the mutant variety lacking the immunodominant epitope.

[0063] Mutant HBsAg may also be provided in the positive control reagent supplied with the immunoassay kit. Such positive controls typically contain HBsAg that has been heat-inactivated and preserved. For example, the positive control of the ETI-MAK-3 assay is a 2.5 mL sample of human recalcified plasma having a maximum concentration of heat-inactivated and preserved HBsAg (subtypes ad and ay) of 0.4-1 U/mL.

[0064] Of course, it should be apparent that the present invention is not intended to be limited to a specific type of diagnostic assay or kit, but rather is intended to include any of a wide variety of diagnostic assays and kits for conducting such assays. Assays can include but are not limited to those based on competition, direct reaction, or sandwich-type assays. Assays may use solid phases or may be performed by immunoprecipitation or any other methods which do not utilize solid phases.

[0065] Assays can take many formats. Some depend upon the use of labeled specific binding molecules such as antibodies, whereas some detect the interaction of antibody (or other specific binding agent) and antigen by observing the resulting precipitation. Examples of qualitative and quantitative assays which do not rely on labeled antibodies include gel precipitation, single radial immunodiffusion, immunoelectrophoresis, including rocket electrophoresis and two-dimensional electrophoresis, and quantification by the scattering of an incident light source (nephelometry). A preferred assay is an agglutination assay; examples of agglutination assays are provided in U.S. Patent Nos. 5,286,452 (Hansen) and 5,589,401 (Hansen et al.).

[0066] Often, some form of labeling is used to detect the antigen-antibody interaction. Labels may be radioactive or non-radioactive. Depending on the format of the assay, either the specific binding agents of the invention can be labeled, or other specific binding agents which bind to them may be labeled. Immunoassays (including radioimmunoassays) and immunometric assays (including immunometric radioassays and enzyme-linked immunosorbent assays) can be used, as can immunoblotting techniques. Chemiluminescent, fluorescent, enzyme, bioluminescent, and epifluorescent labels are also contemplated. IMX assays of the type sold by Abbott Laboratories, Chicago, Illinois, U.S.A., are also contemplated.

[0067] Assays for mutant hepatitis B nucleic acid may also be used. In these methods a nucleic acid probe is used to detect a nucleic acid sequence that codes for the mutant hepatitis B surface antigen protein. Amplification methods such as the polymerase chain reaction may be used in conjunction with the nucleic acid probe. A preferred method of assaying for mutant hepatitis B nucleic acid uses a method known as an DNA enzyme immunoassay (DEIA) in which a probe hybridizes with single-stranded DNA and the hybridized probe-DNA complex is detected with a monoclonal anti-

body that can discriminate between single-stranded and double-stranded DNA. DEIA assays are described in Mantero et al., Clin. Chem., 37/3:422-429 (1991).

[0068] In addition to its use in diagnostic assays, the escape mutant HBsAg of the present invention may also be used in a vaccine to the Hepatitis B virus. In particular, the vaccine can contain an immunogenic amount of the inventive escape mutant in a pharmaceutically acceptable carrier. Immunogenic amounts of the vaccine can be determined using methods well-known to those having ordinary skill in the art. The pharmaceutically acceptable carrier can comprise saline or other suitable carriers, and may also include various additives, adjuvants, and the like. In addition, the vaccine may contain other HBV antigens such as normal HBsAg or composite HBsAg particles containing all or part of the pre-S1 or pre-S2 polypeptides.

#### AMPLIFICATION PROTOCOL

[0069] The "S" gene of HBV has a total length of 657 nucleotides (from nt 157 to nt 837, numbering from the unique EcoR I site of HBV genome); this gene encodes for a protein of 219 amino acids named HBsAg. Two primers were used to perform the amplification reaction and these primers have targets within this range from nt 157 to nt 837.

[0070] The amplification of 45 cycles was performed using the following primers:

primer sense "a4" 5'-CTC GTG GTG GAC TTC TCT CAA TTT-3' (SEQ ID NO:32) (nucleotides 255-278);  
primer antisense "a5" 5'-GGA AAG CCC TAC GAA CCA CTG-3' (SEQ ID NO:33) (nucleotides 717-697).

[0071] The "S" gene fragment amplified had a total length of 463 nt, encoding for a polypeptide of 154 aa (including the amino acid residues 34 and 187 of the HBsAg).

[0072] The composition of the reaction mix was Tris HCl pH=8.8 (67 mM),  $(\text{NH}_4)_2\text{SO}_4$  (1.6 mM),  $\text{MgCl}_2$  (2 mM), 2-mercaptoethanol (10 mM), 100 ug/mL of bovine serum albumin, deoxyribonucleotide triphosphates (dNTPs) (200 uM each), 2.5U of Taq DNA Polymerase (Perkin Elmer), and 50 pmoles of each primer.

[0073] Before amplification, an aliquote of the serum sample was boiled for 5 minutes and then centrifuged at 14000 rpm (4° C) for 15 minutes; the supernatant was recovered and the amplification was carried out in a total volume of 50  $\mu\text{L}$  using 5  $\mu\text{L}$  of the treated sample.

[0074] Each cycle of the amplification, for a total of 45 cycles, included a denaturation step at 94°C for 1 minute, an annealing step at 64°C for 1 minute and an extension step at 72° C for 1 minute; the last cycle had an extension time of 10 minutes to improve the cloning efficiency and the amplification was carried out with a Perkin Elmer Thermal Cycler.

[0075] The amplification product of 463 bp, after purification on agarose gel with Qiaex resin (Qiagen GmbH, Hilden, Germany), was cloned into the pCR2.1 vector (Invitrogen, San Diego, California), transformed in XL-1 blue *E. coli* strain and a subsequent screening was performed on the colonies to identify the positive clones.

[0076] Four clones were chosen to be sequenced, and all of them gave identical results.

[0077] In more detail, the clones was grown overnight at 37° C in LB-broth supplemented with ampicillin; the next day the plasmid DNA was purified from 5 ml of the liquid culture using the Q1A well system (Qiagen GmbH, Hilden, Germany).

[0078] The DNA template prepared in this way, that should be free from protein, chromosomal DNA, and organic solvents, was sequenced using the A.L.F. automated DNA sequencer and the Autoread Sequencing Kit (Pharmacia Biotech, Uppsala, Sweden).

[0079] This system employs a non-radiochemical approach to sequencing, in which a primer labeled with fluorescein at its 5'-terminus is annealed to the template.

[0080] Using standard dideoxy sequencing methods, the fluorescent primer was extended by T7 DNA Polymerase in four separate reactions, and then loaded on polyacrylamide gel.

[0081] The above description and accompanying drawings are provided for the purpose of describing embodiments of the invention and are not intended to limit the scope of the invention in any way. It will be apparent to those skilled in the art that various modifications and variations can be made in the isolated mutant hepatitis B surface antigen proteins, fragments thereof, and particles containing these proteins, specific binding agents thereto, and immunoassays and vaccines using these proteins, fragments thereof, and particles containing these proteins and/or specific binding agents without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

(i) APPLICANTS: Primi, Daniele  
 Fiordalisi, Gianfranco

10 (ii) TITLE OF INVENTION: Escape Mutant of the  
 Surface Antigen of Hepatitis B Virus

(iii) NUMBER OF SEQUENCES: 31

15 (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE:

(B) STREET:

(C) CITY:

20 (D) STATE:

(F) ZIP:

(v) COMPUTER READABLE FORM:

25 (A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 MB  
 Storage

(B) COMPUTER: IBM Compatible Compaq Prolinea  
 4/66

30 (C) OPERATING SYSTEM: Microsoft Windows 95

(D) SOFTWARE: Microsoft Word for Windows  
 Version 7.0

35 (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

40 (C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME:

45 (B) REGISTRATION NUMBER:

(C) REFERENCE/DOCKET NUMBER:

(ix) TELECOMMUNICATION INFORMATION:

50 (A) TELEPHONE:

(B) TELEFAX:

55

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 276 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1

TGT CTG CGG CGT TTT ATC ATC TTC CTC TTC ATC CTG CTG CTA TGC CTC 48  
 Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu  
 1 5 10 15

ATC TTC TTG TTG GCT CTT CTG GAC TAT CAA GGT ATG TTG CCC GTT TGT 96  
 Ile Phe Leu Leu Ala Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys  
 20 25 30

CCT CTA ATT CCA GGA CTC TCG ACC ATC CGC ACG GGA GCA TAC CAA CCC 144  
 Pro Leu Ile Pro Gly Leu Ser Thr Ile Arg Thr Gly Ala Tyr Gln Pro  
 35 40 45

TGC ACG ACT ATT GCT CAA GGA ACC TCT ACG TAT CCC TCC TGT TGC TGT 192  
 Cys Thr Thr Ile Ala Gln Gly Thr Ser Thr Tyr Pro Ser Cys Cys Cys  
 50 55 60

ATC AAA CCT TCG GAC GGA AAT TAC ACC TAT ATT CCC ATC CCA TCA TCC 240  
 Ile Lys Pro Ser Asp Gly Asn Tyr Thr Tyr Ile Pro Ile Pro Ser Ser  
 65 70 75 80

TGG GCT TTC GGA AAA YYC CTA TGG GAG TGG GCC TCA 276  
 Trp Ala Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser  
 85 90

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 92 amino acids  
 (B) TYPE: protein fragment  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2

Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu  
 1 5 10 15  
 Ile Phe Leu Leu Ala Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys  
 20 25 30  
 Pro Leu Ile Pro Gly Leu Ser Thr Ile Arg Thr Gly Ala Tyr Gln Pro  
 35 40 45  
 Cys Thr Thr Ile Ala Gln Gly Thr Ser Thr Tyr Pro Ser Cys Cys Cys  
 50 55 60  
 Ile Lys Pro Ser Asp Gly Asn Tyr Thr Tyr Ile Pro Ile Pro Ser Ser  
 65 70 75 80  
 Trp Ala Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser  
 85 90

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 92 amino acids  
 (B) TYPE: protein fragment  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3

Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu  
 1 5 10 15  
 Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys  
 20 25 30  
 Pro Leu Ile Pro Gly Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr  
 35 40 45  
 Cys Met Thr Thr Ala Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys  
 50 55 60



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Thr Lys Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser  
65 70 75 80

Trp Ala Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser  
85 90

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 92 amino acids

(B) TYPE: protein fragment

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4

Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu  
1 5 10 15

Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys  
20 25 30

Pro Leu Ile Pro Gly Thr Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr  
35 40 45

Cys Thr Ile Pro Ala Gln Gly Thr Ser Met Phe Pro Ser Cys Cys Cys  
50 55 60

Thr Lys Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser  
65 70 75 80

Trp Ala Phe Ala Arg Phe Leu Trp Glu Trp Ala Ser  
85 90

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 92 amino acids

(B) TYPE: protein fragment

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5

Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu  
1 5 10 15

Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys  
                   20                  25                  30  
 5 Pro Leu Ile Pro Gly Ser Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr  
           35                  40                  45  
 Cys Thr Thr Pro Ala Gln Gly Asn Ser Met Phe Pro Ser Cys Cys Cys  
 10          50                  55                  60  
 Ser Lys Pro Thr Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser  
 65                  70                  75                  80  
 15 Trp Ala Phe Gly Lys Tyr Leu Trp Glu Trp Ala Ser  
                   85                  90

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 92 amino acids  
 (B) TYPE: protein fragment  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6

Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu  
 1                  5                  10                  15  
 35 Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys  
           20                  25                  30  
 Pro Leu Ile Pro Gly Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr  
           35                  40                  45  
 40 Cys Thr Thr Pro Ala Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys  
           50                  55                  60  
 Thr Lys Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser  
 45          65                  70                  75                  80  
 Trp Ala Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser  
                   85                  90

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## (2) INFORMATION FOR SEQ ID NO:7:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7

```

Ser Trp Trp Thr Ser Leu Asn Phe Arg Gly Gly Thr Thr Val Cys Leu
1           5           10           15
Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys
20           25           30
Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Gly Phe Ile
35           40           45
Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu
50           55           60
Leu Glu Tyr Gln Gly Met Leu His Val Cys Pro Leu Ile Pro Gly Thr
65           70           75           80
Thr Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln
85           90           95
Gly Asn Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Thr Ser Asp Gly
100          105          110
Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr
115          120          125
Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val
130          135          140
Pro Phe Val Gln Trp Phe Val Gly Leu Ser
145          150

```

## (2) INFORMATION FOR SEQ ID NO:8:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8

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Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu  
 1 5 10 15  
 5 Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
 20 25 30  
 Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
 35 40 45  
 10 Ile Phe Leu Phe Ile Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60  
 Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser  
 65 70 75 80  
 15 Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Thr Thr Pro Ala Gln  
 85 90 95  
 Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser His Gly  
 100 105 110  
 20 Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe  
 115 120 125  
 Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val  
 130 135 140  
 25 Pro Phe Val Gln Trp Phe Met Gly Leu Ser  
 145 150

## (2) INFORMATION FOR SEQ ID NO:9:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu  
 1 5 10 15  
 45 Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
 20 25 30  
 Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
 35 40 45  
 50 Ile Phe Leu Phe Ile Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60

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5 Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser  
 65 70 75 80  
 Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Thr Thr Pro Ala Gln  
 85 90 95  
 Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly  
 100 105 110  
 10 Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe  
 115 120 125  
 Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val  
 130 135 140  
 15 Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
 145 150

20

## (2) INFORMATION FOR SEQ ID NO:10:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu  
 1 5 10 15  
 35 Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
 20 25 30  
 Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
 35 40 45  
 40 Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60  
 Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser  
 65 70 75 80  
 45 Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Thr Thr Pro Ala Gln  
 85 90 95  
 Gly Asn Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly  
 100 105 110  
 50 Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe  
 115 120 125

55

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Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val  
130 135 140

Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
145 150

## (2) INFORMATION FOR SEQ ID NO:11:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu  
1 5 10 15

Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
20 25 30

Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
35 40 45

Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
50 55 60

Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser  
65 70 75 80

Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala Gln  
85 90 95

Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly  
100 105 110

Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe  
115 120 125

Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val  
130 135 140

Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
145 150

# EP 0 919 568 A1

## (2) INFORMATION FOR SEQ ID NO:12:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

```

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu
1          5          10          15
Gly Gln Asn Ser Gln Ser Pro Ile Ser Asn His Ser Pro Thr Ser Cys
20          25          30
Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile
35          40          45
Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu
50          55          60
Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser
65          70          75          80
Ser Thr Thr Ser Thr Gly Ser Cys Arg Thr Cys Thr Thr Pro Ala Gln
85          90          95
Gly Ile Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly
100          105          110
Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe
115          120          125
Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val
130          135          140
Pro Phe Val Gln Trp Phe Val Gly Leu Ser
145          150

```

## (2) INFORMATION FOR SEQ ID NO:13:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

# EP 0 919 568 A1

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13

5 Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu  
 1 5 10 15  
 Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
 20 25 30  
 10 Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Ser Arg Arg Arg Phe Ile  
 35 40 45  
 Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60  
 15 Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser  
 65 70 75 80  
 Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Thr Thr Pro Ala Gln  
 85 90 95  
 Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly  
 100 105 110  
 25 Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe  
 115 120 125  
 Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val  
 130 135 140  
 30 Gly Phe Val Gln Trp Phe Val Gly Leu Ser  
 145 150

## (2) INFORMATION FOR SEQ ID NO:14:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14

5 Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Pro Val Cys Leu  
 1 5 10 15  
 Gly Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys  
 20 25 30



Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
 35 40 45  
 5 Ile Phe Leu Cys Ile Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60  
 Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Leu Gly Ser  
 65 70 75 80  
 10 Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln  
 85 90 95  
 Gly Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Gly  
 100 105 110  
 15 Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr  
 115 120 125  
 Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val  
 130 135 140  
 20 Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
 145 150

25

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 154 amino acids
  - (B) TYPE: protein fragment
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

35

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Pro Val Cys Leu  
 1 5 10 15  
 40 Gly Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys  
 20 25 30  
 Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
 35 40 45  
 45 Ile Phe Leu Cys Ile Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60  
 Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser  
 65 70 75 80  
 50 Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln  
 85 90 95

55

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Gly Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Gly  
 100 105 110

Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr  
 115 120 125

Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val  
 130 135 140

Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
 145 150

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16

Ser Trp Trp Thr Ser Leu Ser Phe Leu Gly Gly Thr Pro Val Cys Leu  
 1 5 10 15

Gly Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys  
 20 25 30

Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
 35 40 45

Ile Phe Leu Cys Ile Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60

Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser  
 65 70 75 80

Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln  
 85 90 95

Gly Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Gly  
 100 105 110

Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr  
 115 120 125

Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val  
 130 135 140

Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
 145 150

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## (2) INFORMATION FOR SEQ ID NO:17:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17

```

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Pro Val Cys Leu
1          5          10          15
Gly Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys
20          25          30
Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile
35          40          45
Ile Phe Leu Cys Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu
50          55          60
Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser
65          70          75          80
Ser Thr Thr Ser Thr Gly Pro Cys Lys Ile Cys Thr Thr Pro Ala Gln
85          90          95
Gly Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Gly
100         105         110
Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr
115         120         125
Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val
130         135         140
Pro Phe Val Gln Trp Phe Val Gly Leu Ser
145         150

```

## (2) INFORMATION FOR SEQ ID NO:18:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

# EP 0 919 568 A1

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18

5 Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Pro Val Cys Leu  
1 5 10 15

Gly Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys  
20 25 30

10 Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
35 40 45

Ile Phe Leu Cys Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
50 55 60

15 Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser  
65 70 75 80

Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln  
85 90 95

20 Gly Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Met Asp Gly  
100 105 110

25 Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr  
115 120 125

Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val  
130 135 140

30 Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
145 150

## (2) INFORMATION FOR SEQ ID NO:19:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19

45 Ser Trp Trp Thr Ser Leu Asn Phe Ile Gly Gly Ser Pro Val Cys Leu  
1 5 10 15

Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
20 25 30

50 Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
35 40 45

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Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
50 55 60

Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser  
65 70 75 80

Thr Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln  
85 90 95

Gly Asn Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Val Gly  
100 105 110

Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr  
115 120 125

Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val  
130 135 140

Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
145 150

## (2) INFORMATION FOR SEQ ID NO:20:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Ser Pro Val Cys Leu  
1 5 10 15

Gly Gln Asn Ser Arg Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
20 25 30

Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
35 40 45

Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
50 55 60

Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Leu Gly Ser  
65 70 75 80

Thr Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln  
85 90 95

Gly Asn Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Gly  
100 105 110

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Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr  
115 120 125

Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu  
130 135 140

Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
145 150

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 154 amino acids

(B) TYPE: protein fragment

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Ser Pro Val Cys Leu  
1 5 10 15

Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
20 25 30

Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
35 40 45

Ile Phe Leu Phe Ile Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
50 55 60

Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Ile Ile Pro Gly Ser  
65 70 75 80

Thr Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln  
85 90 95

Gly Asn Ser Leu Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Gly  
100 105 110

Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr  
115 120 125

Leu Trp Glu Arg Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val  
130 135 140

Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
145 150

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## (2) INFORMATION FOR SEQ ID NO:22:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22

```

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Ala Pro Thr Cys Pro
1           5           10           15
Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys
20           25           30
Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile
20           35           40           45
Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu
50           55           60
Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Leu Pro Gly Thr
25 65           70           75           80
Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Ile Pro Ala Gln
85           90           95
Gly Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly
30 100          105          110
Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Arg Phe
115          120          125
Leu Trp Glu Gly Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val
35 130          135          140
Pro Phe Val Gln Trp Phe Val Gly Leu Ser
40 145          150

```

## (2) INFORMATION FOR SEQ ID NO:23:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23

# EP 0 919 568 A1

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Ala Pro Thr Cys Pro  
 1 5 10 15  
 5 Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
 20 25 30  
 Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
 35 40 45  
 10 Ile Phe Leu Phe Ile Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60  
 Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Leu Pro Gly Thr  
 65 70 75 80  
 15 Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Ile Pro Ala Gln  
 85 90 95  
 Gly Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly  
 100 105 110  
 20 Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Arg Phe  
 115 120 125  
 Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val  
 130 135 140  
 25 Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
 145 150

## (2) INFORMATION FOR SEQ ID NO:24:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Glu Ala Pro Thr Cys Pro  
 1 5 10 15  
 45 Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
 20 25 30  
 Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
 35 40 45  
 50 Ile Phe Leu Phe Ile Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60



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5 Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Leu Pro Gly Thr  
 65 70 75 80  
 Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Ile Pro Ala Gln  
 85 90 95  
 Gly Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly  
 100 105 110  
 10 Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Arg Phe  
 115 120 125  
 Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val  
 130 135 140  
 15 Pro Phe Val Gln Trp Phe Ala Gly Leu Ser  
 145 150

20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 154 amino acids  
 (B) TYPE: protein fragment  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Ala Pro Thr Cys Pro  
 1 5 10 15  
 35 Gly Arg Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys  
 20 25 30  
 Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
 35 40 45  
 40 Ile Phe Leu Phe Ile Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60  
 Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Leu Pro Gly Thr  
 65 70 75 80  
 45 Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln  
 85 90 95  
 Gly Asn Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly  
 100 105 110  
 50 Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Arg Phe  
 115 120 125

55

Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val  
 130 135 140

Pro Phe Val Gln Trp Phe Val Gly Leu Ser  
 145 150

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 154 amino acids

(B) TYPE: protein fragment

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Leu Pro Gly Cys Pro  
 1 5 10 15

Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Leu Pro Thr Ser Cys  
 20 25 30

Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile  
 35 40 45

Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu  
 50 55 60

Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Leu Pro Gly Ser  
 65 70 75 80

Thr Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Leu Ala Gln  
 85 90 95

Gly Thr Ser Met Phe Pro Ser Cys Cys Cys Ser Lys Pro Ser Asp Gly  
 100 105 110

Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Leu Gly Lys Tyr  
 115 120 125

Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val  
 130 135 140

Gln Phe Val Gln Trp Cys Val Gly Leu Ser  
 145 150

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27

```

Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu
1           5           10           15
Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys
20           25           30
Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile
35           40           45
Ile Phe Leu Phe Ile Leu Leu Cys Leu Ile Phe Leu Leu Ala Leu
50           55           60
Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Leu
65           70           75           80
Ser Thr Thr Arg Thr Gly Ala Tyr Gln Pro Cys Thr Thr Ile Ala Gln
85           90           95
Gly Thr Ser Thr Tyr Pro Ser Cys Cys Cys Ile Lys Pro Ser Asp Gly
100          105          110
Asn Tyr Thr Tyr Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe
115          120          125
Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val
130          135          140
Pro Phe Val Gln Trp Phe Ala Gly Leu Ser
145          150

```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28

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Arg Thr Gly Ala Tyr Gln Pro  
1 5

5

## (2) INFORMATION FOR SEQ ID NO:29:

### (i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH: 21 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29

Leu Ser Thr Ile Arg Thr Gly Ala Tyr Gln Pro Cys Thr Thr Ile Ala  
1 5 10 15

20

Gln Gly Thr Ser Thr  
20

## (2) INFORMATION FOR SEQ ID NO:30:

25

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30

35

Ile Lys Pro Ser Asp Gly Asn Tyr Thr Tyr  
1 5 10

40

## (2) INFORMATION FOR SEQ ID NO:31:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 amino acids
- (B) TYPE: protein fragment
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

45

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31

50

Arg Thr Gly Ala Tyr Gln Pro Cys Thr Thr Ile Ala Gln Gly Thr Ser  
1 5 10 15

55

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Thr Tyr Pro Ser Cys Cys Cys Ile Lys Pro Ser Asp Gly Asn Tyr Thr  
20 25 30

5 Tyr

(2) INFORMATION FOR SEQ ID NO:32:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 bases

(B) TYPE: nucleic acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32

20 CTCGTGGTGG ACTTCTCTCA ATTT

(2) INFORMATION FOR SEQ ID NO:33:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 bases

(B) TYPE: nucleic acid

30 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33

35 GGAAAGCCCT ACGAACCACT G

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 bases

45 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34

GGAYTMTCGA CCATCMGCAC GGGAGCATAC CAACCC

55

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35

GGGTTGGTAT GCTCCCGTGC KGATGGTCGA KARTCC

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36

ACCATCMGCA CGGGAGCATA CCAA

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37

TTGGTATGCT CCCGTGCKGA TGGT

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

EP 0 919 568 A1

- (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38

CCAGGAYTMT CGACCATCMG CACG

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39

TCCCGTGCKG ATGGTCGAKA RTCC

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40

GCATACCAAC CCTGCACGAC TATTG

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

EP 0 919 568 A1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41

5

CAATAGTCGT GCAGGGTTGG TATGC

10

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 bases

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42

20

GTATCAAACC TTCGGACGGA AATTACACCT ATA

25

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 bases

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43

35

TCGGACGGAA ATTACACCTA TA

40

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 bases

45

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44

50

TGGGATGGGA ATATAGGTGT AA

55



# Claims

1. An isolated mutant hepatitis B surface antigen protein which comprises an amino acid sequence of a surface antigen protein of hepatitis B virus which infects humans, in which the amino acid at position 121 is not cysteine and at least one of the amino acids at positions 120, 122, 123, 147, or 149 is not a conserved amino acid for its position.
2. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein displays an antigenicity different from that of wild-type hepatitis B surface antigen protein.
3. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein at least one of the amino acids at positions 120, 122, or 123 is not a conserved amino acid for its position.
4. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein at least one of the amino acids at positions 141 or 149 is not a conserved amino acid for its position.
5. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein at least two of the amino acids at positions 120, 122, 123, 147, or 149 are not conserved amino acids for their positions.
6. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein at least three of the amino acids at positions 120, 122, 123, 147, or 149 are not conserved amino acids for their positions.
7. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein at least four of the amino acids at positions 120, 122, 123, 147, or 149 are not conserved amino acids for their positions.
8. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein all five amino acids at positions 120, 122, 123, 147, or 149 are not conserved amino acids for their positions.
9. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the conserved cysteine at position 121 is replaced by tyrosine.
10. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the conserved proline at position 120 is replaced by alanine.
11. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the conserved lysine or arginine at position 122 is replaced by glutamine.
12. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the conserved threonine at position 123 is replaced by proline.
13. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the conserved proline at position 120 is replaced by alanine, the conserved lysine or arginine at position 122 is replaced by glutamine, and the conserved threonine at position 123 is replaced by proline.
14. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acid at position 127 is not a conserved amino acid for its position.
15. An isolated mutant hepatitis B surface antigen protein of claim 14, wherein the conserved proline at position 127 is replaced by isoleucine.
16. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acid at position 117 is not a conserved amino acid for its position.
17. An isolated mutant hepatitis B surface antigen protein of claim 16, wherein the conserved serine at position 117 is replaced by arginine.
18. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acid at position 96 is not a conserved amino acid for its position.

19. An isolated mutant hepatitis B surface antigen protein of claim 18, wherein the conserved valine at position 96 is replaced by alanine.
20. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acid at position 113 is not a conserved amino acid for its position.
21. An isolated mutant hepatitis B surface antigen protein of claim 20, wherein the conserved serine or threonine at position 113 is replaced by leucine.
22. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acid at position 133 is not a conserved amino acid for its position.
23. An isolated mutant hepatitis B surface antigen protein of claim 22, wherein the conserved methionine at position 133 is replaced by threonine.
24. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acid at position 140 is not a conserved amino acid for its position.
25. An isolated mutant hepatitis B surface antigen protein of claim 24, wherein the conserved threonine at position 140 is replaced by isoleucine.
26. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acid at position 147 is not a conserved amino acid for its position.
27. An isolated mutant hepatitis B surface antigen protein of claim 26, wherein the conserved cysteine at position 147 is replaced by tyrosine.
28. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acid at position 149 is not a conserved amino acid for its position.
29. An isolated mutant hepatitis B surface antigen protein of claim 28, wherein the conserved cysteine at position 149 is replaced by tyrosine.
30. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acids at positions 147 and 149 are not conserved amino acids for their positions.
31. An isolated mutant hepatitis B surface antigen protein of claim 30, wherein the conserved cysteines at position 121, 147, and 149 are replaced by tyrosines.
32. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acid at position 145 is the conserved glycine.
33. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acids at positions 137, 138, and 139 are all conserved cysteines.
34. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the amino acid at position 124 is the conserved cysteine.
35. An isolated mutant hepatitis B surface antigen protein which comprises an amino acid sequence of a surface antigen protein of hepatitis B virus which infects humans, in which the amino acid at position 122 is not a conserved amino acid for its position.
36. An isolated mutant hepatitis B surface antigen protein of claim 35, wherein the protein displays an antigenicity different from that of wild-type hepatitis B surface antigen protein.
37. An isolated mutant hepatitis B surface antigen protein of claim 35, wherein the conserved lysine or arginine at position 122 is replaced by glutamine.

38. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein is the major protein of hepatitis B surface antigen.
39. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein is the middle protein of hepatitis B surface antigen.
40. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein is the large protein of hepatitis B surface antigen.
41. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein includes a pre-S sequence.
42. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein includes a portion of or a complete pre-S1 sequence.
43. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein includes a portion of or a complete pre-S2 sequence.
44. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein comprises the following sequence of amino acids at positions 117 to 123: Arg-Thr-Gly-Ala-Tyr-Gln-Pro (SEQ ID NO: 28).
45. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein comprises the following sequence of amino acids at positions 113 to 133: Leu-Ser-Thr-Ile-Arg-Thr-Gly-Ala-Tyr-Gln-Pro-Cys-Thr-Thr-Ile-Ala-Gln-Gly-Thr-Ser-Thr (SEQ ID NO:29).
46. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein comprises the following sequence of amino acids at positions 140 to 147: Ile-Lys-Pro-Ser-Asp-Gly-Asn-Tyr-Thr-Tyr (SEQ ID NO:30).
47. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein comprises the following sequence of amino acids at positions 117 to 123: Arg-Thr-Gly-Ala-Tyr-Gln-Pro (SEQ ID NO:28) and the following sequence of amino acids at positions 140 to 147: Ile-Lys-Pro-Ser-Asp-Gly-Asn-Tyr-Thr-Tyr (SEQ ID NO:30).
48. An isolated mutant hepatitis B surface antigen protein of claim 1, wherein the protein comprises the following sequence of amino acids at positions 117 to 149: Arg-Thr-Gly-Ala-Tyr-Gln-Pro-Cys-Thr-Thr-Ile-Ala-Gln-Gly-Thr-Ser-Thr-Tyr-Pro-Ser-Cys-Cys-Cys-Ile-Lys-Pro-Ser-Asp-Gly-Asn-Tyr-Thr-Tyr (SEQ ID NO:31).
49. An isolated mutant hepatitis B surface antigen protein of claim 1 which comprises the sequence of amino acids listed in SEQ ID NO:2.
50. An isolated mutant hepatitis B particle which comprises the mutant hepatitis B surface antigen protein of one of claims 1 to 49.
51. An isolated fragment of a mutant hepatitis B surface antigen protein of claim 1 in which the isolated fragment comprises a sequence of at least thirty amino acids, said sequence comprising the amino acids at positions 120 to 149, in which the amino acid at position 121 is not cysteine and at least one of the amino acids at positions 120, 122, 123, 147, or 149 is not a conserved amino acid for its position.
52. An isolated fragment of a mutant hepatitis B surface antigen protein of claim 35 in which the isolated fragment comprises a sequence of at least thirty amino acids, said sequence comprising the amino acids at positions 120 to 149, in which the amino acid at position 122 is not a conserved amino acid for its position.
53. A composition which comprises a mutant hepatitis B surface antigen protein of one of claims 1 or 35, a fragment thereof, or a particle containing the protein, and is substantially blood-free.
54. A vaccine which comprises an immunogenic amount of a mutant hepatitis B surface antigen protein of one of claims 1 or 35, a fragment thereof, or a particle containing the protein, in a pharmaceutically acceptable carrier.
55. A specific binding agent which specifically recognizes a mutant hepatitis B surface antigen protein of one of claims 1 or 35, a fragment thereof, or a particle containing the protein, wherein the specific binding agent was specifically

raised against the mutant hepatitis B surface antigen protein, a fragment thereof, or a particle containing the protein.

56. A specific binding agent of claim 55, wherein the specific binding agent recognizes a sequential epitope.

57. A specific binding agent of claim 55, wherein the specific binding agent recognizes a conformational epitope.

58. A specific binding agent of claim 55, wherein the specific binding agent is a monoclonal antibody.

59. A specific binding agent of claim 55, wherein the specific binding agent is a fragmentary monoclonal antibody.

60. A specific binding agent of claim 55, wherein the specific binding agent is a polyclonal antibody.

61. A secreting hybridoma of a monoclonal antibody of claim 58.

62. A control sample comprising a known amount of a mutant hepatitis B surface antigen protein of one of claims 1 or 35, a fragment thereof, or a particle containing the protein.

63. A method for detecting in a sample a mutant hepatitis B surface antigen protein or a particle containing the protein comprising:

(a) contacting a sample with a specific binding agent which specifically recognizes a mutant surface antigen protein of one of claims 1 or 35 under conditions suitable for binding to occur; and

(b) measuring the extent of the binding of the specific binding agent, wherein the extent of the binding correlates to the presence or amount of mutant hepatitis B surface antigen protein or a particle containing the protein in the sample;

to determine the presence or amount of mutant hepatitis B surface antigen protein or a particle containing the protein in the sample.

64. A method of claim 63, wherein the correlation between the extent of the binding and the presence or amount of mutant hepatitis B surface antigen protein or a particle containing the protein in the sample has been determined using control samples containing known amounts of the mutant hepatitis B surface antigen protein, a fragment thereof, or a particle containing the protein.

65. A method of claim 63, wherein the specific binding agent was specifically raised against the mutant hepatitis B surface antigen protein or a particle containing the protein.

66. A method of claim 63, wherein the method comprises a non-competitive assay.

67. A method of claim 66, wherein the assay comprises a second specific binding agent which has a label.

68. A method of claim 67, wherein the label is a radioactive label, an enzyme label, a fluorescent label, a chemiluminescent label, a bioluminescent label, or an epifluorescent label.

69. A method of claim 63, wherein the method comprises an agglutination assay.

70. A method of claim 63, wherein the method comprises a competitive assay.

71. A method of claim 63, wherein the method comprises using a labeled substrate that cross-reacts with the mutant hepatitis B surface antigen protein or particle containing the protein.

72. A method of claim 63, wherein the sample is a blood sample.

73. A method of claim 63, wherein the sample is a vaccine.

74. A method for calibrating an assay for a mutant hepatitis B surface antigen protein or a particle containing the protein comprising:

- (a) providing two or more control samples comprising known amounts of a mutant hepatitis B surface antigen protein of one of claims 1 or 35, a fragment thereof, or a particle containing the protein;
- (b) testing each of the control samples in the assay and recording the signal that each control sample produced; and
- (c) producing a calibration curve that correlates the signal produced with the amount of mutant hepatitis B surface antigen protein, the fragment thereof, or the particle containing the protein in a sample.

75. A method of testing the binding affinity of a specific binding agent comprising:

- (a) providing a control sample comprising a known amount of a mutant hepatitis B surface antigen protein of one of claims 1 or 35, a fragment thereof, or a particle containing the protein;
  - (b) contacting the control sample with a specific binding agent under conditions suitable for binding to occur; and
  - (c) measuring the extent of the binding of the specific binding agent;
- to determine the binding affinity of the specific binding agent to the mutant hepatitis B surface antigen protein, the fragment thereof, or the particle containing the protein.

76. A method of claim 75, further comprising comparing the binding affinity with a predetermined minimum value for the binding affinity.

77. A method of claim 76, wherein the method is part of a quality control procedure for producing a diagnostic immunoassay kit.

78. A method of claim 75, wherein the specific binding agent is a monoclonal antibody or a fragmentary monoclonal antibody.

79. A diagnostic immunoassay kit comprising a specific binding agent of claim 55.

80. A diagnostic immunoassay kit comprising a control sample of claim 62.

81. A mutant hepatitis B surface antigen protein of one of claims 1 or 35, a fragment thereof, or a particle containing the protein, which has been recombinantly produced.

82. A protein, particle, or fragment of claim 81, which has been recombinantly produced in a yeast, bacterium, or mammalian cell.

83. Isolated DNA which codes for a mutant hepatitis B surface antigen protein of one of claims 1 or 35, a fragment thereof, or a particle containing the protein.

84. Isolated DNA of claim 83 which comprises a sequence which codes for the amino acid sequence of SEQ ID NO: 2.

85. Isolated DNA of claim 83 which comprises a sequence which codes for the following amino acid sequence: Arg-Thr-Gly-Ala-Tyr-Gln-Pro-Cys-Thr-Thr-Ile-Ala-Gln-Gly-Thr-Ser-Thr-Tyr-Pro-Ser-Cys-Cys-Cys- Ile-Lys-Pro-Ser-Asp-Gly-Asn-Tyr-Thr-Tyr (SEQ ID NO:31).

86. A vector comprising DNA which codes for a hepatitis B surface antigen protein of one of claims 1 or 35, a fragment thereof, or a particle containing the protein.

87. A method of detecting in a sample a nucleic acid sequence coding for a mutant hepatitis B surface antigen protein comprising:

- (a) providing a sample suspected of containing a nucleic acid sequence coding for a mutant hepatitis B surface antigen protein of one of claims 1 or 35; and
- (b) detecting the nucleic acid sequence coding for the mutant hepatitis B surface antigen protein.

88. A method of claim 87, wherein prior to the detecting step (b) at least a portion of the nucleic acid sequence is amplified.

89. A method of claim 88, wherein the detecting step is performed by hybridizing the amplified nucleic acid with at least a portion of a nucleotide probe comprising a nucleotide sequence complementary to the amplified mutant hepatitis B nucleic acid; and detecting the probe.

5 90. A method of claim 89, wherein the probe is labeled.

91. A method of claim 89, wherein the hybridized probe and amplified nucleic acid complex is detected by a specific binding agent which is specific to double-stranded DNA.

10 92. A method of claim 91, wherein the specific binding agent is a monoclonal antibody or a fragmentary monoclonal antibody.

93. A method of claim 88, wherein the detecting step is performed by separating the amplified nucleic acid according to nucleotide sequence length and detecting nucleotide sequences corresponding to amplified hepatitis B nucleic acid.

15 94. A method of claim 89, wherein the nucleotide probe comprises a nucleotide sequence selected from:

5'-GGA YTM TCG ACC ATC MGC ACG GGA GCA TAC CAA CCC-3';

20 5'-GGG TTG GTA TGC TCC CGT GCK GAT GGT CGA KAR TCC-3';

5'-ACC ATC MGC ACG GGA GCA TAC CAA-3';

25 5'-TTG GTA TGC TCC CGT GCK GAT GGT-3';

5'-CCA GGA YTM TCG ACC ATC MGC ACG-3';

30 5'-TCC CGT GCK GAT GGT CGA KAR TCC-3';

5'-GCA TAC CAA CCC TGC ACG ACT ATT G-3';

5'-C AAT AGT CGT GCA GGG TTG GTA TGC-3';

35 5'-GT ATC AAA CCT TCG GAC GGA AAT TAC ACC TAT A-3';

5'-TCG GAC GGA AAT TAC ACC TAT A-3';

40 5'-TGG GAT GGG AAT ATA GGT GTA A-3';

or a nucleotide sequence having substantially the same nucleotide sequence and having substantially the same hybridization activity of any of the listed nucleotide sequences.

45 95. A nucleotide probe for detection of a nucleic acid sequence coding for a mutant hepatitis B surface antigen protein of one of claims 1 or 35 comprising a nucleotide sequence selected from:

5'-GGA YTM TCG ACC ATC MGC ACG GGA GCA TAC CAA CCC-3';

50 5'-GGG TTG GTA TGC TCC CGT GCK GAT GGT CGA KAR TCC-3';

5'-ACC ATC MGC ACG GGA GCA TAC CAA-3';

5'-TTG GTA TGC TCC CGT GCK GAT GGT-3';

55 5'-CCA GGA YTM TCG ACC ATC MGC ACG-3';

5'-TCC CGT GCK GAT GGT CGA KAR TCC-3';

5'-GCA TAC CAA CCC TGC ACG ACT ATT G-3';

5'-C AAT AGT CGT GCA GGG TTG GTA TGC-3';

5 5'-GT ATC AAA CCT TCG GAC GGA AAT TAC ACC TAT A-3';

5'-TCG GAC GGA AAT TAC ACC TAT A-3';

10 5'-TGG GAT GGG AAT ATA GGT GTA A-3';

or a nucleotide sequence having substantially the same nucleotide sequence and having substantially the same hybridization activity of any of the listed nucleotide sequences.

96. A nucleotide probe of claim 95, further comprising a detectable label.

15

97. A nucleotide probe of claim 95, wherein the probe consists essentially of one of the listed sequences.

98. A kit for the detection of a nucleic acid sequence coding for a mutant hepatitis B surface antigen protein of one of claims 1 or 35 comprising:

20

at least one reagent comprising nucleotide primer capable of annealing to and priming for amplification with nucleic acid characteristic for hepatitis B surface antigen; and  
at least one reagent comprising a nucleotide probe capable of hybridizing with the amplified mutant hepatitis B surface antigen nucleic acid.

25

99. A kit of claim 98, wherein the nucleotide probe comprises a nucleotide sequence selected from:

5'-GGA YTM TCG ACC ATC MGC ACG GGA GCA TAC CAA CCC-3';

30

5'-GGG TTG GTA TGC TCC CGT GCK GAT GGT CGA KAR TCC-3';

5'-ACC ATC MGC ACG GGA GCA TAC CAA-3';

5'-TTG GTA TGC TCC CGT GCK GAT GGT-3';

35

5'-CCA GGA YTM TCG ACC ATC MGC ACG-3';

5'-TCC CGT GCK GAT GGT CGA KAR TCC-3';

40

5'-GCA TAC CAA CCC TGC ACG ACT ATT G-3';

5'-C AAT AGT CGT GCA GGG TTG GTA TGC-3';

5'-GT ATC AAA CCT TCG GAC GGA AAT TAC ACC TAT A-3';

45

5'-TCG GAC GGA AAT TAC ACC TAT A-3';

5'-TGG GAT GGG AAT ATA GGT GTA A-3';

50

or a nucleotide sequence having substantially the same nucleotide sequence and having substantially the same hybridization activity of any of the listed nucleotide sequences.

55

CLRRFIIFLFILLCLIFLLALLDYQGMLPVCPLIPGLSTIRTGAYQPCTTIAQ  
GTSTYPSCCCIKPSDGNYTYIPIPSWAFGKFLWEWAS (SEQ ID NO:2)

Fig. 1



HBVAYW3  
 CLRRFIIFLFILLLLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCM 50  
 HBVADR  
 CLRRFIIFLFILLLLCLIFLLVLLDYQGMLPVCPLIPGTSTTSTGPCKTCT 50  
 HBVADW 2  
 CLRRFIIFLFILLLLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCKTCT 50  
 HBVAYW  
 CLRRFIIFLFILLLLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCT 50  
 CTS  
 CLRRFIIFLFILLLLCLIFLLALLDYQGMLPVCPLIPGLSTIRTGAYQPCT 50

HBVAYW3  
 TTAQGTSMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWAS 92  
 (SEQ ID NO:3)  
 HBVADR  
 IPAQGTSMFPSCCCTKPSDGNCTCIPIPSSWAFARFLWEWAS 92  
 (SEQ ID NO:4)  
 HBVADW2  
 TPAQGNMFPSCCCKPTDGNCTCIPIPSSWAFGKYLWEWAS 92  
 (SEQ ID NO:5)  
 HBVAYW  
 TPAQGTSMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWAS 92  
 (SEQ ID NO:6)  
 CTS  
 TIAQGTSTYPSCCCIKPSDGNYTYIPIPSSWAFGKFLWEWAS 92  
 (SEQ ID NO:2)

Fig. 2

7AYWS  
 SWWTSLNFRGGTTVCLGQNSQSPTSNSHSPTSCPPTCPGYRWMCLRGFIIF 50  
 6AYWS  
 SWWTSLNFLGGTTVCLGQNSQSPTSNSHSPTSCPPICPGYRWMCLRRFIIF 50  
 3AYWS  
 SWWTSLNFLGGTTVCLGQNSQSPTSNSHSPTSCPPTCPGYRWMCLRRFIIF 50  
 2AYWS  
 SWWTSLNFLGGTTVCLGQNSQSPTSNSHSPTSCPPTCPGYRWMCLRRFIIF 50  
 1AYWS  
 SWWTSLNFLGGTTVCLGQNSQSPTSNSHSPTSCPPTCPGYRWMCLRRFIIF 50  
 1ADYWS  
 SWWTSLNFLGGTTVCLGQNSQSPTSNSHSPTSCPPTCPGYRWMCLRRFIIF 50  
 5AYWS  
 SWWTSLNFLGGTTVCLGQNSQSPTSNSHSPTSCPPTCPGYRWMSRRRFIIF 50  
 5ADWS  
 SWWTSLNFLGGTPVCLGQNSQSQISSHSPTCCPPICPGYRWMCLRRFIIF 50  
 2ADWS  
 SWWTSLNFLGGTPVCLGQNSQSQISSHSPTCCPPICPGYRWMCLRRFIIF 50  
 3ADWMUT  
 SWWTSLSFLGGTPVCLGQNSQSQISSHSPTCCPPICPGYRWMCLRRFIIF 50  
 2ADWMUT  
 SWWTSLNFLGGTPVCLGQNSQSQISSHSPTCCPPICPGYRWMCLRRFIIF 50  
 1ADWS  
 SWWTSLNFLGGTPVCLGQNSQSQISSHSPTCCPPICPGYRWMCLRRFIIF 50  
 4ADWMUT  
 SWWTSLNFIGGSPVCLGQNSQSPTSNSHSPTSCPPICPGYRWMCLRRFIIF 50  
 3ADWS  
 SWWTSLNFLGGSPVCLGQNSRSPTSNSHSPTSCPPICPGYRWMCLRRFIIF 50  
 1ADWMUT  
 SWWTSLNFLGGSPVCLGQNSQSPTSNSHSPTSCPPICPGYRWMCLRRFIIF 50  
 4ADRS  
 SWWTSLNFLGGAPTCPGQNSQSPTSNSHSPTSCPPICPGYRWMCLRRFIIF 50  
 1ADRS  
 SWWTSLNFLGGAPTCPGQNSQSPTSNSHSPTSCPPTCPGYRWMCLRRFIIF 50

Fig. 3A

3ADRS  
 SWWTSLNFLGEAPTCPGQNSQSPTSNSHSPTSCPPICPGYRWMCLRRFIIF 50  
 2ADRS  
 SWWTSLNFLGGAPTCPGRNSQSPTSNSHSPTSCPPICPGYRWMCLRRFIIF 50  
 4ADWS  
 SWWTSLNFLGGLPGCPGQNSQSPTSNSHLPTSCPPTCPGYRWMCLRRFIIF 50  
 CTS  
 SWWTSLNFLGGTTVCLGQNSQSPTSNSHSPTSCPPTCPGYRWMCLRRFIIF 50  
 \*\*\*\*\*.\* \*. . \* \*.\*\*.\*..\* \*\*.\*\*\*.\*\*\*\*\*\*. \* \*\*\*\*

7AYWS  
 LFILLCLIFLLVLLDYQGMLHVCPLIPGTTTTSTGPCKTCTTPAQNSM 100  
 6AYWS  
 LFILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSM 100  
 3AYWS  
 LFILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSM 100  
 2AYWS  
 LFILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQNSM 100  
 1AYWS  
 LFILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCMTTAQGTSM 100  
 1ADYWS  
 LFILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGSCRTCTTPAQGISM 100  
 5AYWS  
 LFILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSM 100  
 5ADWS  
 LCILLCLIFLLVLLDYQGMLPVCPLILGSSTTSTGPCKTCTTPAQGTSM 100  
 2ADWS  
 LCILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCKTCTTPAQGTSM 100  
 3ADWMUT  
 LCILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCKTCTTPAQGTSM 100  
 2ADWMUT  
 LCILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCKICTTPAQGTSM 100

Fig. 3B

1ADWS  
 LCILLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCKTCTTPAQGTSM 100  
 4ADWMUT  
 LFILLCLIFLLVLLDYQGMLPVCPLIPGSTTTSTGPCKTCTTPAQGNSM 100  
 3ADWS  
 LFILLCLIFLLVLLDYQGMLPVCPLILGSTTTSTGPCKTCTTPAQGNSM 100  
 1ADWMUT  
 LFILLCLIFLLVLLDYQGMLPVCPIIPGSTTTSTGPCKTCTTPAQGNSL 100  
 4ADRS  
 LFILLCLIFLLVLLDYQGMLPVCPLLPGTSTTSTGPCKTCTIPAQGTSM 100  
 1ADRS  
 LFILLCLIFLLVLLDYQGMLPVCPLLPGTSTTSTGPCKTCTIPAQGTSM 100  
 3ADRS  
 LFILLCLIFLLVLLDYQGMLPVCPLLPGTSTTSTGPCKTCTIPAQGTSM 100  
 2ADRS  
 LFILLCLIFLLVLLDYQGMLPVCPLLPGTSTTSTGPCKTCTTPAQGNSM 100  
 4ADWS  
 LFILLCLIFLLVLLDYQGMLPVCPLLPGSTTTSTGPCKTCTTLAQGTSM 100  
 CTS  
 LFILLCLIFLLALLDYQGMLPVCPLIPGLSTIRTGAYQPCTTIAQGTST 100  
 \* \*\*\*\*\*.\*.\*.\*.\*.\*.\*.\* \* .\*.\*\*\* \* . \*\*\* \*

7AYWS  
 FPSCCCTKTS DGNCTCIPIPSSWAFKYLWEWASVRFSWLSLLVPFVQWF 150  
 6AYWS  
 YPSCCCTKPSHG NCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWF 150  
 3AYWS  
 YPSCCCTKPSDG NCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWF 150  
 2AYWS  
 YPSCCCTKPSDG NCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWF 150  
 1AYWS  
 YPSCCCTKPSDG NCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWF 150  
 1ADYWS  
 YPSCCCTKPSDG NCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWF 150

Fig. 3C

5AYWS  
 YPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVGFVQWF 150  
 5ADWS  
 FPSCCCTKPTDGNCTCIPIPSSWAFKYLWEWASVRFSWLSLLVPFVQWF 150  
 2ADWS  
 FPSCCCTKPTDGNCTCIPIPSSWAFKYLWEWASVRFSWLSLLVPFVQWF 150  
 3ADWMUT  
 FPSCCCTKPTDGNCTCIPIPSSWAFKYLWEWASVRFSWLSLLVPFVQWF 150  
 2ADWMUT  
 FPSCCCTKPTDGNCTCIPIPSSWAFKYLWEWASVRFSWLSLLVPFVQWF 150  
 1ADWS  
 FPSCCCTKPM DGNCTCIPIPSSWAFKYLWEWASVRFSWLSLLVPFVQWF 150  
 4ADWMUT  
 FPSCCCTKPTVGNCTCIPIPSSWAFKYLWEWASVRFSWLSLLVPFVQWF 150  
 3ADWS  
 FPSCCCTKPTDGNCTCIPIPSSWAFKYLWEWASVRFSWLSLLVPFVQWF 150  
 1ADWMUT  
 FPSCCCTKPTDGNCTCIPIPSSWAFKYLWERASVRFSWLSLLVPFVQWF 150  
 4ADRS  
 FPSCCCTKPSDGNCTCIPIPSSWAFARFLWEGASVRFSWLSLLVPFVQWF 150  
 1ADRS  
 FPSCCCTKPSDGNCTCIPIPSSWAFARFLWEWASVRFSWLSLLVPFVQWF 150  
 3ADRS  
 FPSCCCTKPSDGNCTCIPIPSSWAFARFLWEWASVRFSWLSLLVPFVQWF 150  
 2ADRS  
 FPSCCCTKPSDGNCTCIPIPSSWAFARFLWEWASVRFSWLSLLVPFVQWF 150  
 4ADWS  
 FPSCCCKPSDGNCTCIPIPSSWALGKYLWEWASARFSWLSLLVQFVQWC 150  
 CTS  
 YPSCCCKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWF 150  
 .\*\*\*\*\* \* . \*\*\*\*\* .....\*\*\* \*\* .\*\*\*\*\* \*\*\*\*\*

Fig. 3D

7AYWS	VGLS	154	(SEQ ID NO:7)
6AYWS	MGLS	154	(SEQ ID NO:8)
3AYWS	VGLS	154	(SEQ ID NO:9)
2AYWS	VGLS	154	(SEQ ID NO:10)
1AYWS	VGLS	154	(SEQ ID NO:11)
1ADYWS	VGLS	154	(SEQ ID NO:12)
5AYWS	VGLS	154	(SEQ ID NO:13)
5ADWS	VGLS	154	(SEQ ID NO:14)
2ADWS	VGLS	154	(SEQ ID NO:15)
3ADWMUT	VGLS	154	(SEQ ID NO:16)
2ADWMUT	VGLS	154	(SEQ ID NO:17)
1ADWS	VGLS	154	(SEQ ID NO:18)
4ADWMUT	VGLS	154	(SEQ ID NO:19)
3ADWS	VGLS	154	(SEQ ID NO:20)
1ADWMUT	VGLS	154	(SEQ ID NO:21)
4ADRS	VGLS	154	(SEQ ID NO:22)
1ADRS	VGLS	154	(SEQ ID NO:23)
3ADRS	AGLS	154	(SEQ ID NO:24)
2ADRS	VGLS	154	(SEQ ID NO:25)
4ADWS	VGLS	154	(SEQ ID NO:26)
CTS	AGLS	154	(SEQ ID NO:27)

\*\*\*

Fig. 3E

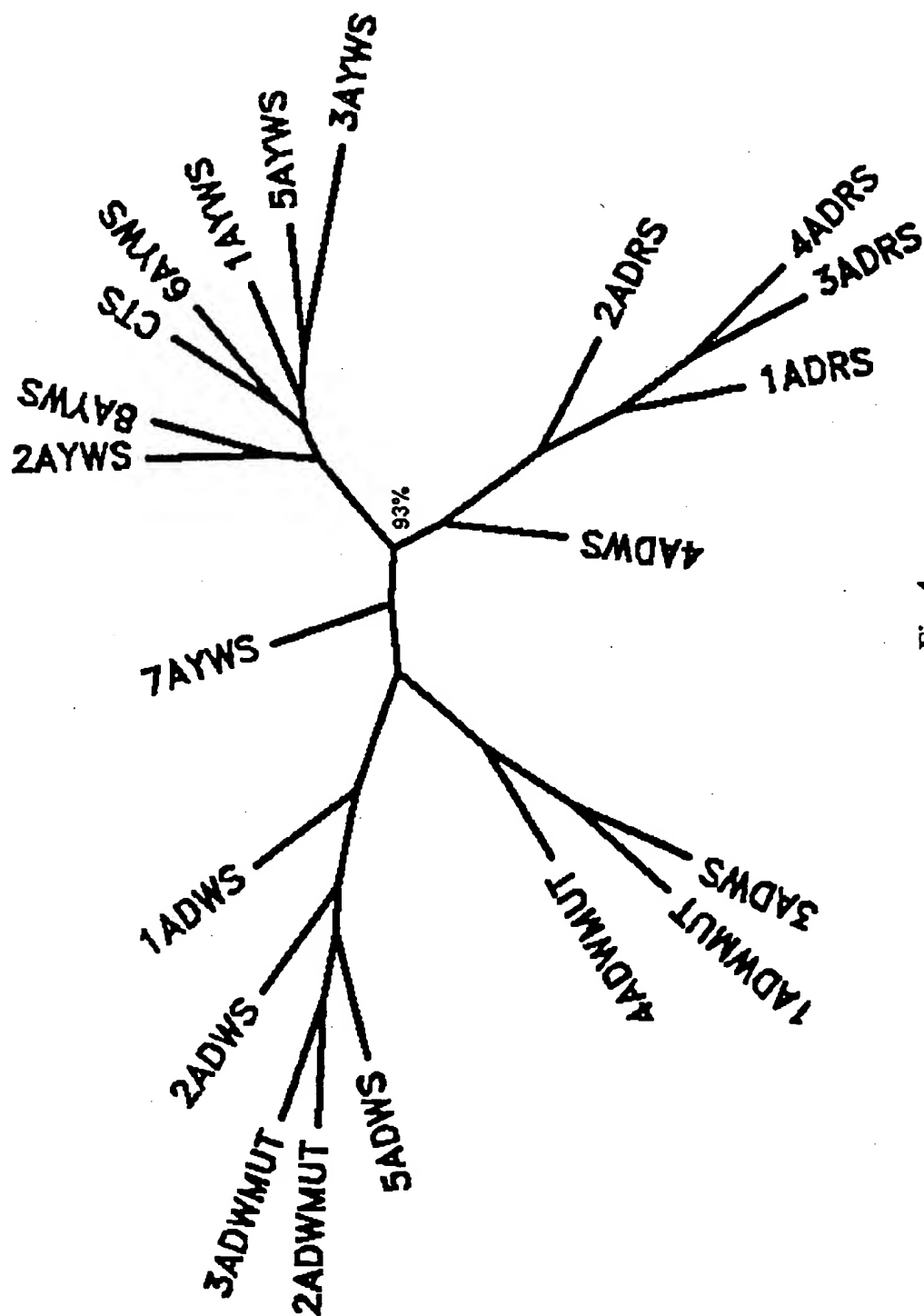


Fig. 4

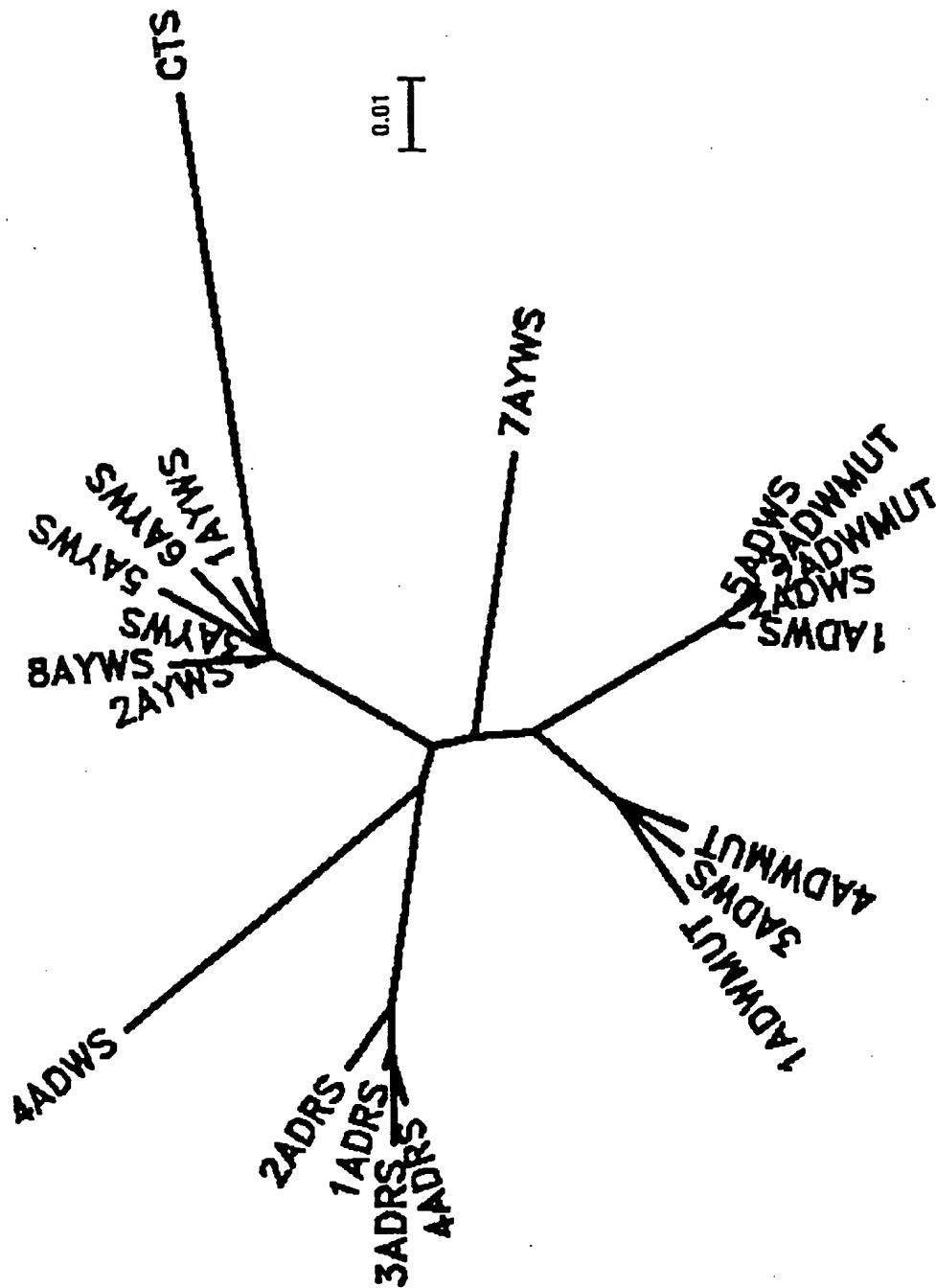


Fig. 5



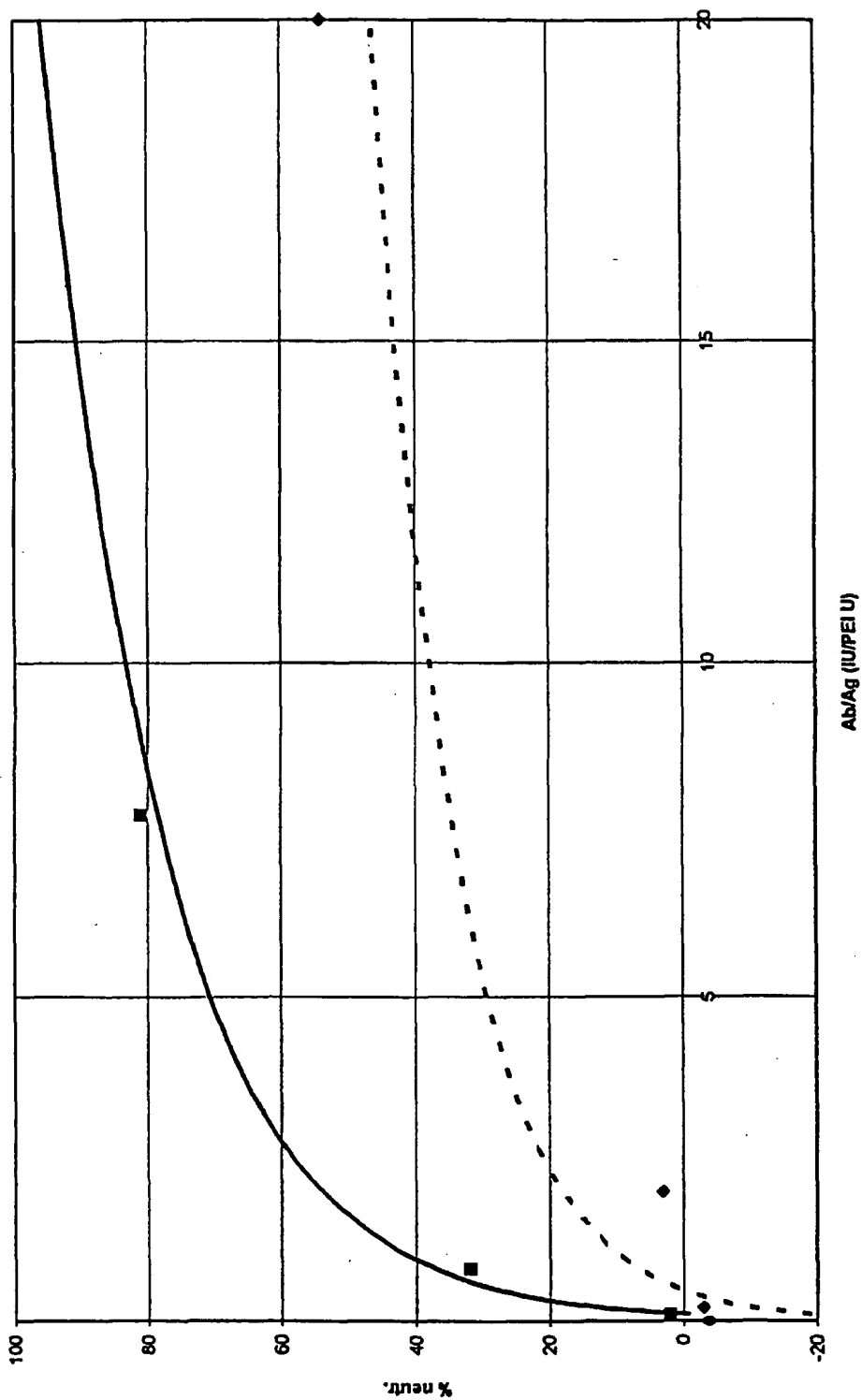


Fig. 6

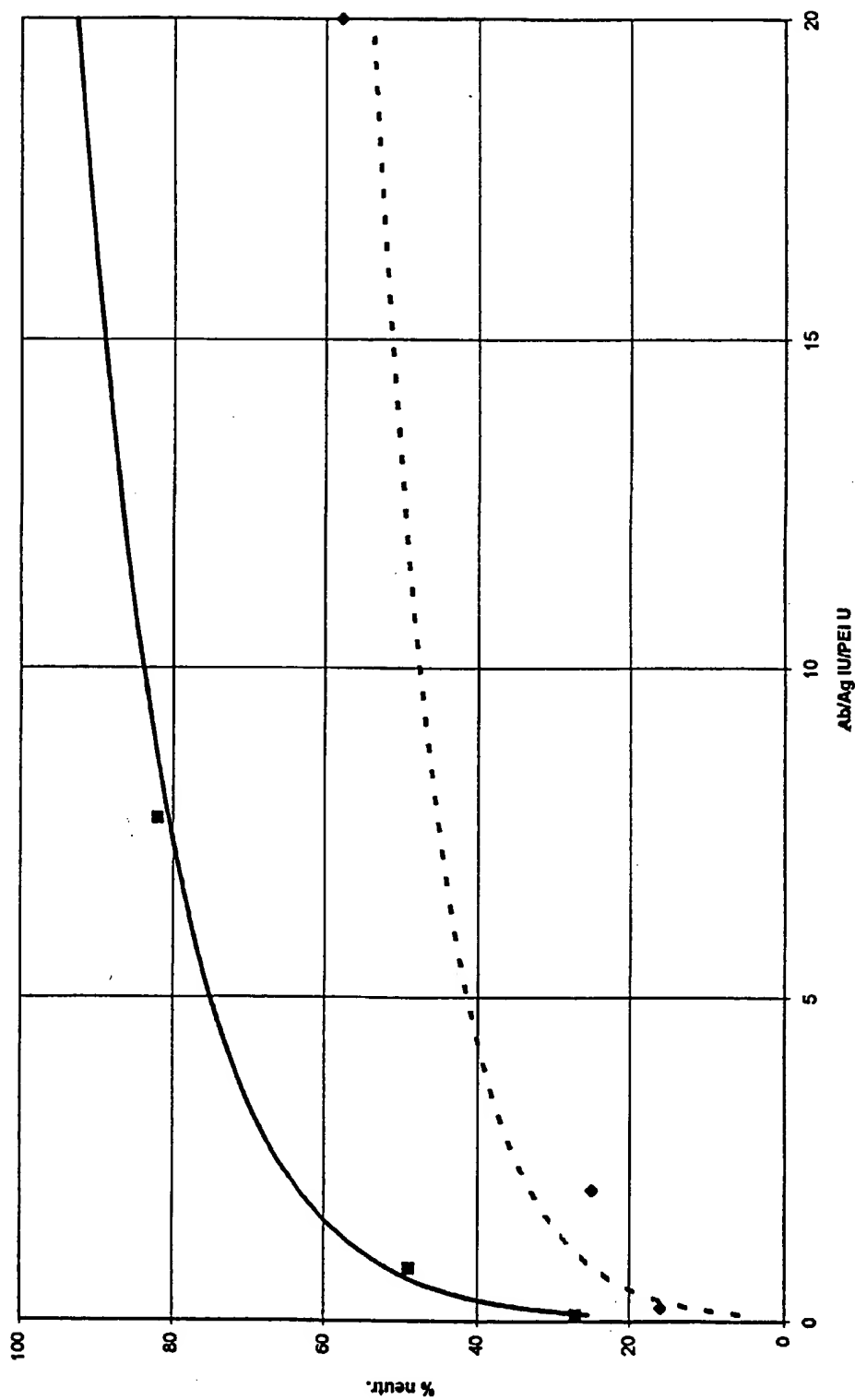
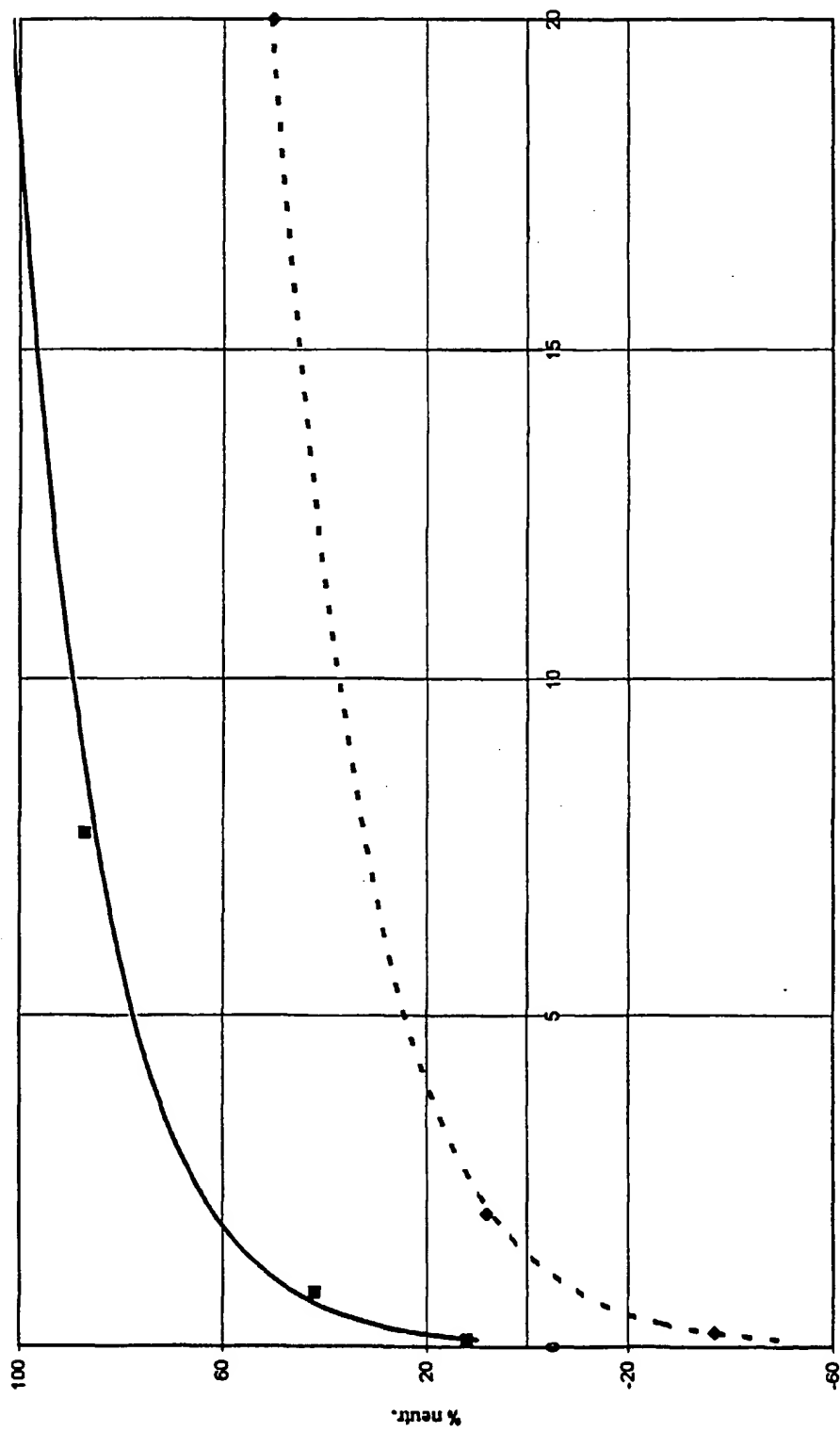


Fig. 7



Abvag (IU/PEI U)

Fig. 8



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# PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 97 83 0635  
shall be considered, for the purposes of subsequent  
proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	WO 87 06594 A (SOUTHWEST FOUNDATION FOR BIOMEDICAL RESEARCH) 5 November 1987	1,2,4	C07K14/02 A61K39/29 C12Q1/68 G01N33/576
Y	* examples VI-IX *	1-99	
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X	WO 97 39029 A (ABBOTT LABORATORIES) 23 October 1997	35,36	
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D,A	MANGOLD, C.M.T. ET AL.: "Secretion and antigenicity of Hepatitis B virus small envelope proteins lacking cysteines in the major antigenic region" VIROLOGY, vol. 211, 1995, pages 535-543, XP002072491 * table 1 *	1-99	
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			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C07K A61K C12Q G01N
<b>INCOMPLETE SEARCH</b>			
<p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely : 1-52,63,65-73,84,85,87-99</p> <p>Claims searched incompletely : 53-62,64,74-83,86</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search:</p> <p>Claims 53-62,64,74-83,86 refer to undefined fragments of proteins or nucleic acids; note that a single amino acid or a single nucleotide are "fragments" of a larger entity.</p>			
Place of search <b>MUNICH</b>		Date of completion of the search <b>23 March 1999</b>	Examiner <b>Hermann, R</b>
CATEGORY OF CITED DOCUMENTS		<p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>&amp; : member of the same patent family, corresponding document</p>	
<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p>			

EPO FORM 1503 03 82 (P04C07)



European Patent  
Office

## PARTIAL EUROPEAN SEARCH REPORT

Application Number  
EP 97 83 0635

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D,A	--- CARIANI, E. ET AL.: "Emergence of hhepatitis B virus S gene mutant in a liver transplant recipient" J. MED. VIROL., vol. 47, 1995, pages 410-415, XP002072493 * fig. 2 *	1-99	
X	--- WO 94 26904 A (ABBOTT LABORATORIES) 24 November 1994	35,36, 52-83, 86-99	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
Y	* claims *	1-99	
X	--- WO 94 25486 A (LUCKY LIMITED) 10 November 1994 * p.26-28;see especially primer PE2AXHO * -----	95,99	



European Patent  
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LACK OF UNITY OF INVENTION  
SHEET B

Application Number  
EP 97 83 0635

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-34, 38-51, 84-85 completely; 53-83,  
86-99 partially (see also "incomplete search")

HBsAgs in which the amino acid at position 121 is not cysteine and at least one of the amino acids at positions 120, 122, 123, 147, or 149 is not a conserved amino acid for this position; related subject-matter.

2. Claims: 35-37, 52 completely; 53-83,  
86-99 partially (see also "incomplete search")

HBsAgs in which the amino acid at position 121 is cysteine and in which the amino acids at position 122 is not a conserved amino acid for this position; related subject-matter

3. Claims: 94-97, 99 (partially)

Probes which are not directed to the region around Cys121/Lys122 (NOTE: only probes with SEQ ID NOs 34, 36, 37, 40, 41 detect said region); related subject-matter.

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 97 83 0635

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

23-03-1999

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